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REVIEW

Inter-relationships between gastric emptying and glycaemia: Implications for clinical practice

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Abstract

Gastric emptying (GE) exhibits a wide inter-individual variation and is a major determinant of postprandial glycaemia in health and diabetes; the rise in blood glucose following oral carbohydrate is greater when GE is relatively more rapid and more sustained when glucose tolerance is impaired. Conversely, GE is influenced by the acute glycaemic environment acute hyperglycaemia slows, while acute hypoglycaemia accelerates it. Delayed GE (gastroparesis) occurs frequently in diabetes and critical illness. In diabetes, this poses challenges for management, particularly in hospitalised individuals and/or those using insulin. In critical illness it compromises the delivery of nutrition and increases the risk of regurgitation and aspiration with consequent lung dysfunction and ventilator dependence. Substantial advances in knowledge relating to GE, which is now recognised as a major determinant of the magnitude of the rise in blood glucose after a meal in both health and diabetes and, the impact of acute glycaemic environment on the rate of GE have been made and the use of gut-based therapies such as glucagon-like peptide-1 receptor agonists, which may profoundly impact GE, in the management of type 2 diabetes, has become commonplace. This necessitates an increased understanding of the complex inter-relationships of GE with glycaemia, its implications in hospitalised patients and the relevance of dysglycaemia and its management, particularly in critical illness. Current approaches to management of gastroparesis to achieve more personalised

diabetes care, relevant to clinical practice, is detailed. Further studies focusing on the interactions of medications affecting GE and the glycaemic environment in hospitalised patients, are required.

Key Words: Glycaemia; Gastric emptying; Clinical practice; Glucagon-like peptide-1

Core Tip: Gastric emptying (GE) is a major determinant of postprandial glycaemia in health, diabetes and critical illness. Acute hyperglycaemia slows GE while insulin-induced hypoglycaemia accelerates it. Gastroparesis occurs frequently in diabetes and critical illness with a weak correlation between gastrointestinal symptoms and GE. Accordingly, diagnosis of gastroparesis should ideally be made after measuring GE with an optimal technique. Glucagon-like peptide-1 receptor agonists, commonly used in the treatment of type 2 diabetes and increasingly in obesity, may profoundly impact GE. We explore the rationale for current glycaemic targets and the implications of dysglycaemia and its management in hospitalised and critically ill populations.

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INTRODUCTION

In recent years there has been increasing interest regarding the relevance of gastrointestinal (GI) function, particularly gastric emptying (GE), to post-prandial glycaemia. GE is now recognised as a major determinant of the magnitude of the rise in blood glucose after a meal in both health and diabetes [1,2]. Moreover, in the past decade, use of gut-based therapies such as glucagon-like peptide-1 (GLP-1) receptor agonists (RAs), which may profoundly impact GE, in the management of type 2 diabetes, has become commonplace. On the other hand, it is also clear that the acute glycaemic environment impacts the rate of GE. This review focuses on two inter-related areas: Current knowledge of GE, including the pathophysiology of gastroparesis, and the inter-relationships between GE and glycaemia, including the clinical implications of these insights in hospitalised patients with diabetes, and for critical illness.

GI SYMPTOMS IN DIABETES

Although GI symptoms occur frequently in the general community[3], they are much more prevalent in people with diabetes and the consequences are generally underappreciated, despite impacting quality of life negatively[4]. These symptoms can be classified based on their apparent predominant site of origin in the GI tract, such as from the oesophagus (reflux, dysphagia), stomach (nausea/vomiting, bloating, abdominal distension, early satiety, abdominal pain and discomfort) or the intestines (diarrhoea, constipation, faecal incontinence)[5]. Epidemiological studies are indicative of a wide, but consistently high, prevalence (between 40% to 80%) of upper GI symptoms in people with diabetes, particularly females, the obese, those with *Helicobacter Pylori* infection and the elderly[5]. It is uncertain whether the prevalence differs between type 1 and type 2 diabetes. The natural history of GI symptoms remains poorly characterized, but a substantial turnover (*i.e.*, appearance and disappearance of symptoms over time) has been observed. The latter may be to the order of 25% over a 24-mo period, such that the overall prevalence appears to be relatively constant[6]. A number of validated questionnaires for the assessment of GI symptoms, including the Patient Assessment of Upper Gastrointestinal Symptom Severity Index[7] and the Diabetes Bowel Symptom Questionnaire, are available but unfortunately, many clinical trials, particularly those related to glucose-lowering therapies[8] continue to report GI symptoms/adverse effects relying solely on participant self-reporting, which is known to be unreliable [9]. An important concept that is still poorly appreciated is that the association of upper GI symptoms with GI motility, including the rate of GE is generally weak in people with diabetes[9-11]. Therefore, a diagnosis of GI dysmotility (including gastroparesis) should not rely on symptoms alone and necessitates objective measurement.

GE

GE exhibits a wide inter-individual variability (approximately 1-4 kcal/min) in health, which is even greater in type 2 diabetes. A substantial proportion of people with longstanding, complicated type 2 diabetes (40%) have gastroparesis whereas, in uncomplicated type 2 diabetes[12] and adolescents with type 1 diabetes[13], GE is often abnormally accelerated. It should be appreciated that in patients with gastroparesis, the magnitude of the delay in GE is often modest[14]. The prevalence of delayed GE in ambulant people with diabetes remains uncertain, particularly as the diagnosis has been based primarily on the presence of significant upper GI symptoms, but diabetes appears to be the most common cause of gastroparesis[15]. The techniques currently available for measurement of GE are summarized in Figure 1.

Physiology of normal GE

The principal function of the stomach is transient storage, breakdown and transportation of ingested food. Patterns of gastroduodenal motility are distinct between the fasting and fed states. In the fasted state, a characteristic pattern is observed, referred to as the migratory motor complex (MMC) which has a 'house-keeping' role to propagate residual or undigested food through the GI tract[16]. The MMC, which lasts approximately 85-110 min comprises four, distinct phases: The first phase is quiescent (approximately 45-60 min) in which there are no contractions, the second involves initiation of intermittent and irregular contractions, the latter become stronger and more regular with bursts in the third phase, with each burst lasting for 5-15 min and occurring periodically every approximately 90-120 min. The fourth is a transitory period of irregular contractions between the third phase and the quiescent first phase. Thus, the MMC prepares the stomach for the arrival of food, by clearing its content[17]. The MMC continues until nutrients (liquid or solid) are ingested, when it is replaced by continuous post-prandial contractile activity. An important function of the stomach is to 'accommodate' the ingested food from the oesophagus with minimal increase in intra-gastric pressure, facilitated by a reduction in gastric tone and increase in compliance after meal ingestion[18]. As food moves from the proximal to the distal stomach, larger solid food particles are ground, predominantly in the antrum, into a fine chyme (partly digested semi-solid contents of the stomach) consisting of particles 1-2 mm in size which are delivered into the small intestine[19].

The rate of GE is regulated primarily by inhibitory feedback arising from the interaction with receptors in the small intestine, rather than intragastric factors[20], The magnitude of this feedback is dependent on both the region and length of small intestine exposed. GE involves a coordinated interplay of the extrinsic nervous system (mediated by the vagus), intrinsic or enteric nervous system (comprising Auerbach's or myenteric, which controls the rate of peristalsis and Meissner's plexus located below the level of the musculature, which controls secretion into the lumen of digestive tract), neurotransmitters [both excitatory *e.g.*, acetylcholine and substance-P and inhibitory *e.g.*, nitric oxide (NO) and VIP], the interstitial cells of Cajal (ICCs), mesenchymal cells including platelet-derived growth factors-alpha + cells, fibroblasts, haem-oxygenase 1, macrophages *etc.*[14,21], immune and smooth muscle cells. Gastric accommodation is mediated, at least in part, by the inhibitory neurotransmitter NO, while antral contractility is modulated by the excitatory neurotransmitter acetylcholine[22,23]. The ICCs are densely located in the corpus and antrum of the stomach, within the Auerbach plexus and regarded as 'pacemakers' for GI motility[24] by generating slow-waves responsible for contractions[25] and acting as mechanosensors by affecting the resting membrane potential through nitrergic and cholinergic transmission[26]. The ICCs act as a bridge between the extrinsic nervous system and the enteric nervous system to facilitate smooth muscle contraction.

Pathophysiology of disordered GE

Abnormally delayed GE, or gastroparesis, is generally a chronic disorder which can be defined as delayed emptying of nutrients from the stomach in the absence of mechanical obstruction[25]. The most common causes of gastroparesis are diabetes, post-surgical and idiopathic. The pathophysiology of disordered GE is, not surprisingly, multifactorial. Significant advances have been made in the last decade and a half, in part, due to concerted efforts of the National Institutes of Health funded, Gastroparesis Clinical Research Consortium (GpCRC). Autonomic neuropathy is mainly responsible for gastroparesis and vagal dysfunction is believed to contribute[27]. At the cellular level, a hallmark feature of gastroparesis is a reduction in the ICC[14]. GpCRC data indicates that in 50% of those with diabetic gastroparesis there is a reduction in ICC[28] and even when there is not a reduction, there are abnormalities in the ICC[28], so that the majority of these cells show signs of apoptosis, with increased mast cells and altered nerve endings which are either large or empty[29]. Expression of neuronal NO synthase[30] is reduced in diabetic gastroparesis[29]. The Kit receptor, tyrosine kinase, is expressed in ICC and loss of the receptor is characteristic in delayed GE[14]. In some studies this has been observed to be associated with a reduction in macrophages and their expression of Haeme-oxygenase 1, potentially affecting the capacity for repair and anti-inflammatory response in these cells[14] as well as increasing their susceptibility to oxidative damage. The heterogenous nature of the dysfunctions in gastroparesis has major implications for effective management.

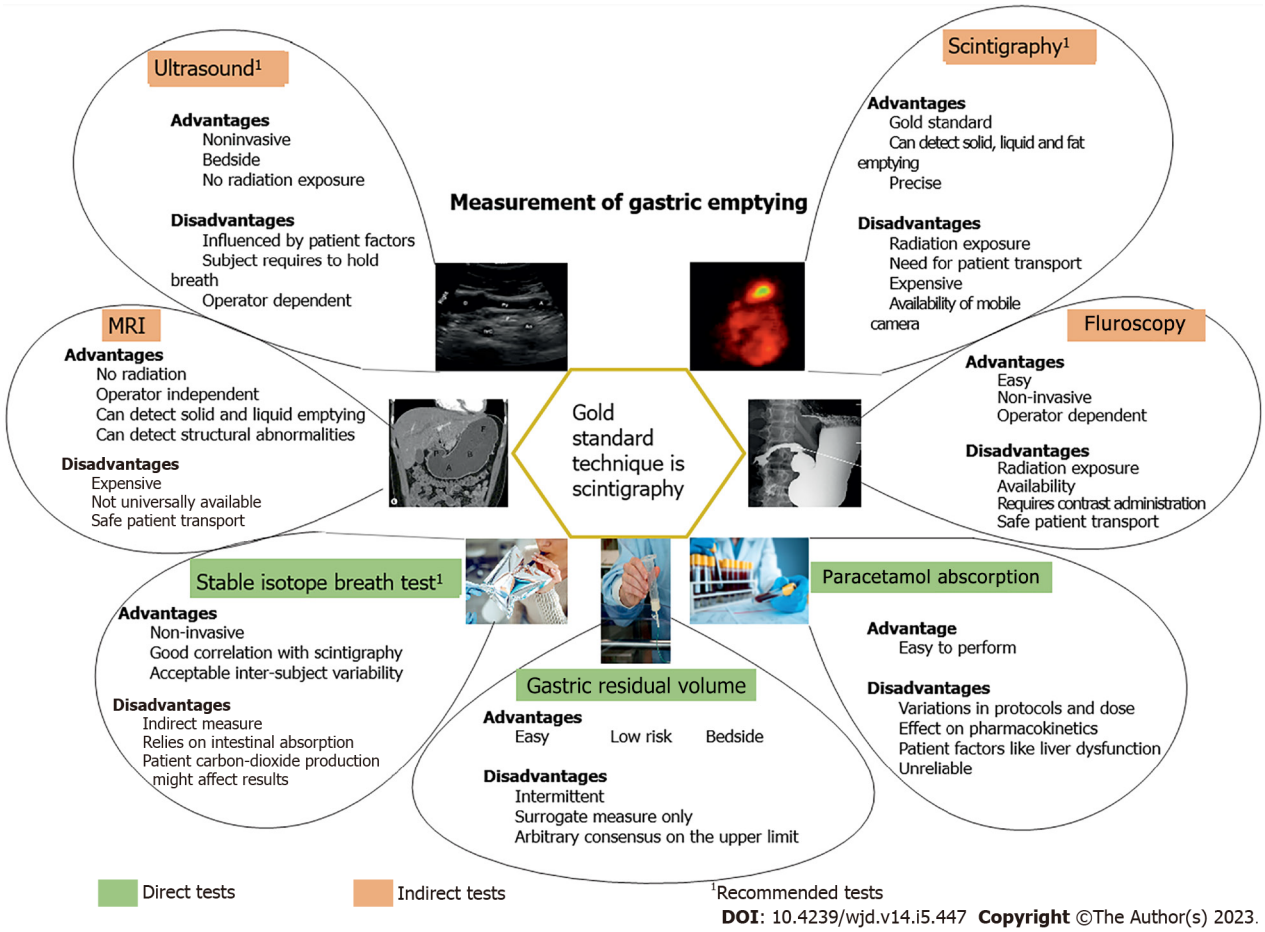

Figure 1 Measurement of gastric emptying.

Relationship between GE and glycaemia

The rate of GE is both a determinant of, as well as determined by, acute changes in glycaemia. Accordingly, studies exploring the impact on glycaemia have tended to control the rate of GE (*e.g.*, by use of naso-duodenal infusions) and those exploring the impact on rate of emptying have controlled the glycaemic level (usually withglucose-insulin clamps). These studies are thus experimental in nature and the conclusions should be regarded as 'proof-of-principle'. There is less information about the impact of spontaneous fluctuations in blood glucose.

The impact of GE on glycaemia: There are number of determinants of post-prandial glycaemia, including pre-prandial glycaemia, endogenous glucose production (hepatic and renal), intestinal glucose absorption and its disposal by the liver, hormone secretion (incretins, insulin) and insulin sensitivity[31]. GE, is now recognised to account for almost 35% of the variance in the initial post-prandial glycaemic response in both health[1] and type 2 diabetes[32]. In individuals with normal glucose tolerance, GE of a 75 g oral glucose drink is directly related to the 'initial' *i.e.* 30 min, plasma glucose, not 60 min and inversely related to the blood glucose at 120 min[1]. In contrast, in individuals with impaired glucose tolerance and type 2 diabetes, the rate of GE is related directly to glycaemia at 30 and 60 min and, particularly in type 2 diabetes, there is also a direct relationship at 120 min (the blood glucose level used in the diagnosis of diabetes) with a relatively faster GE associated with an increased glycaemic response, indicative of a 'rightward' shift[33,34].

There is evidence that in insulin-treated patients delayed GE/gastroparesis predisposes to post-prandial hypoglycaemia by inducing a mismatch in the coordination of nutrient delivery with the systemic availability of insulin we have proposed the term "gastric hypoglycaemia" to describe this phenomenon[35]. A Japanese study reported that in type 1 patients with gastroparesis, on continuous subcutaneous (SC) insulin infusion therapy, there was a reduction in the post-prandial insulin requirement in the first 120 min, and a greater requirement between 180-240 min[36]. A community study from Israel reported that GE was delayed in the majority of patients (approximately 80%) with unexplained hypoglycaemia[37]. The effect of accelerating/normalising GE on glycaemic control in these groups is not known and warrants evaluation.

The impact of glycaemia on GE: As mentioned, studies evaluating the impact of acute changes in glycaemia on emptying have largely relied on experimental models, particularly the so-called glucose-

insulin 'clamp' technique. These have shown that acute hyperglycaemia slows GE of nutrient containing meals in health and type 1 diabetes, an effect which is dependent on the level of glycaemia[38-41]. Even so-called "physiological" hyperglycaemia (*i.e.* approximately 8 mmol/L), compared to 4 mmol/L slows GE in health[42] and type 1 diabetes[41]. Hebbard *et al*[43] studied regional stomach motility in health and showed that acute hyperglycaemia (15 mmol/L) affected proximal gastric motor function[43] while Samsom *et al*[40] studied antroduodenal motility using manometry in patients with type 1 diabetes and evidence of autonomic neuropathy and demonstrated a reduction in post-prandial antral contractility during hyperglycaemia (16-19 mmol/L)[40]. Acute hyperglycaemia also appears to delay GE in type 2 diabetes[44] and critically ill[45,46]. In contrast, spontaneous fluctuations in glycaemia has none, or a lesser effect on GE [47].

The impact of chronic glycaemia, as assessed by glycated haemoglobin (HbA1c) on GE is poorly defined, including the effect of improved glycaemic control. Analysis of the data from the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) study[48] indicates that delayed GE is associated with abnormal measures of longer-term hyperglycaemia, such as HbA1c[49]. The impact of intensive glucose lowering on GE is uncertain. Laway *et al*[50] studied asymptomatic women with newly diagnosed type 2 diabetes and reported a substantial acceleration of GE with improved glycaemic control, but the design of the study was uncontrolled[50]. Other studies failed to find any effect of improved glycaemic control[51]. Bharucha *et al*[49], followed up participants from the DCCT[49] and its subsequent follow-up (DCCT-EDIC)[48] and found that those with a longer duration of diabetes and worse glycaemic control at baseline, tended to have delayed GE. However, because GE was not quantified at baseline, the impact of intensive glucose-lowering on GE could not be evaluated. The outcomes of other retrospective studies evaluating the relationship of chronic glycaemia (based on HbA1c) and GE are inconsistent[52-54]. Accordingly, further studies are required.

While there is less information about the effects of acute insulin-induced hypoglycaemia on GE, the outcomes are more consistent. Hypoglycaemia is the most common and feared symptom of insulin, and sulfonylurea, treated diabetes and represents a major limiting factor in achieving optimal glucose control[55]. In response to an acute reduction in blood glucose, a predictable sequence of protective (counter-regulatory) mechanisms are elicited in health[56]. Most widely recognised are the hormonal counter-regulatory responses (early response modulated by glucagon and catecholamines and later responses by cortisol and GH)[57,58]. It is not well appreciated that acute hypoglycaemia also accelerates GE markedly. As early as 1924, *i.e.* within 3 years of the commercial availability of insulin, Bulatao and Carson[59] reported increased contractions of the fasting canine stomach after insulin administration and attributed this effect to hypoglycaemia. In the 1990s and 2000s, acceleration of GE was confirmed employing the 'gold standard' technique of scintigraphy to measure GE, in both health and type 1 diabetes. We recently showed that the magnitude of acceleration of GE is also dependent on the level of the hypoglycaemia in health GE was accelerated during both mild; Approximately 3.6 mmol/L (approximately 20% difference) and marked; Approximately 2.6 mmol/L (40% difference) hypoglycaemia when compared to euglycaemia; approximately 6 mmol/L, but was faster during marked compared with mild hypoglycaemia[42]. This acceleration of GE, which is still evident in type 1 patients with gastroparesis or cardiovascular autonomic neuropathy[56]. This acceleration of GE, which is still evident in type 1 patients with gastroparesis and/or cardiovascular autonomic neuropathy[60], is likely to be an important counter-regulatory mechanism which supports more rapid intestinal glucose absorption[57]. Studies evaluating the effects of hypoglycaemia on GE in the critically ill are, not surprisingly, lacking because of the established harmful effects of hypoglycaemia in this population[61, 62].

Relevance of the insights of the GE-glycaemia relationships to clinical situations

The management of dysglycaemia and its consequences in hospitalised patients is of more relevance due to increasing prevalence of diabetes in this group. The implications of the use of the newer anti-diabetic medications in this group is also of substantial interest.

HOSPITAL (NON-CRITICAL CARE SETTING)

Dysglycaemia is a major issue in hospitalised patients and associated with poor outcomes, including increased length of stay, morbidity and mortality[63].The prevalence of diabetes is markedly higher in hospitalised patients when compared to the community ranging from 22%-46%[60,64]. While hyperglycaemia is a well-recognised poor prognostic indicator, hypoglycaemia has been reported to occur in about 6% of hospitalised patients[64]. There is only limited information about the relationship of GE to dysglycaemia in this group.

Gastroparesis in hospitalised diabetic patients

GE is seldom measured using an optimal technique in the hospital setting unless gastroparesis is

suspected. Iatrogenic aetiologies (due to medications or post-surgery) are also common. Nevertheless, the prevalence of delayed GE measured with scintigraphy has been estimated to be between 17% to 30% [65] in hospitalised patients with diabetes. Kojecky *et al*[65] found that female gender, nausea and early satiety were associated with a higher probability of delayed GE[65]. The impact of medications affecting GI motility (*e.g.*, anticholinergics, sympathomimetic vasopressors, GLP-1RAs, opioids, prokinetics *etc.*) on drug and nutrient absorption during hospitalization is not known. While it is intuitively likely that undiagnosed gastroparesis will increase morbidity in hospitalised patients, there is lack of information about this.

GLP-1 based therapies in the management of type-2 diabetes

The gut-derived incretin hormones (GIP and GLP-1) account for about 50% of the post-prandial insulin response in health[66,67] and are responsible for the 'incretin effect' [the amplified insulin secretory response to oral compared with intravenous (IV) glucose]. GIP is the dominant incretin in health[68] but its insulinotropic capacity is markedly attenuated in type 2 diabetes[69], unlike GLP-1, which largely retains the insulin stimulating and glucagon supressing properties. The rate of GE impacts the secretion of incretin hormones. Studies employing intraduodenal glucose infusion, an experimental model for estimating the impact of GE on incretin secretion by bypassing the gastric pylorus, suggest that there may be a 'threshold' rate of emptying at which significant GLP-1 release is observed following a carbohydrate containing meal[70]. Increasing the rate of intraduodenal glucose infusion from 1 to 4 kcal/min results in a proportionate increase in GIP release; in contrast there is minimal, if any GLP-1 release with an infusion rate < 2 kcal/min, with sustained responses at 3 and 4 kcal/min[71].

Native GLP-1, located primarily in the distal small intestine and triggered following macronutrient exposure, is degraded within minutes *in vivo*, by the ubiquitous enzyme, dipeptidyl peptidase IV (DPP-IV). Two strategies: (1) DPP-IV inhibition which prevents degradation of the enzyme; and (2) GLP-1RAs have been developed to exploit GLP-1 pharmaceutically. Both classes of medication are widely available but the use of GLP-1RA's, in particular, is expanding rapidly (approximately $11.3 billion global sales in 2019, projected to grow to approximately $18.2 billion by 2027). Recent, large-scale, cardiovascular and renal outcome studies have shown positive benefits of these agents particularly in individuals with diabetes and concomitant ischaemic heart disease or cardiac failure[72].

GLP-1RAs are, in nearly all cases, administered by SC injection either daily or weekly. GLP-1RAs, especially the 'short acting' agents, such as exenatide BD and lixisenatide, primarily act by delaying GE and thereby reducing post-prandial glycaemia[73], while the effect of 'long-acting' GLP-1RA's (*e.g.*, dulaglutide, semaglutide) has been poorly characterised due to the use of suboptimal methodology (paracetamol absorption)[74,75]. It had been assumed that they had no effect with sustained use due to tachyphylaxis, but it is now clear that both the exenatide once weekly preparation and liraglutide do slow GE[76,77] and there are anecdotal reports of retained gastric content at endoscopy with these drugs[78]. The effects of these drugs on small intestinal transit, which may affect carbohydrate absorption are poorly studied. Long-acting GLP-1RAs are used increasingly to induce weight loss in obese individuals.

A fundamental issue with these agents is their current essentially empirical use. Given its central importance, the effect of these drugs on GE should be characterised; it is likely that they all slow GE; patients taking long-acting GLP-1RAs for type 2 diabetes or obesity (higher dose) should be, accordingly, regarded at increased risk for delayed GE (*i.e.*, gastroparesis), until this is shown not to be the case, whereas the effect of short-acting GLP-1RA's should be transient, reflecting their plasma half-life. The impact of GLP-1RA on GE in different glycaemic environments (such as acute hyperglycaemia or hypoglycaemia) is not known. While GLP-1RA by themselves seldom cause hypoglycaemia (*i.e.*, their actions are glucose-dependent)[79], in combination with insulin or sulphonylureas, there is an increased risk of hypoglycaemia. There is need for further studies evaluating the effect of long-acting GLP-1RAs in the presence of other medications that affect GE (prokinetics, oral opioid pain medications *etc.*). In contrast, DPP-IV inhibitors have minimal or no impact on GE[80], presumably because of the more modest elevation in GLP-1. However, the rate of GE influences the post-prandial glycaemic response to DPP-IV inhibitors[81].

HOSPITAL (CRITICAL CARE SETTING)

Dysglycaemia is also common in critically ill patients, can present as hyperglycaemia, hypoglycaemia or glycaemic variability and is associated with increased mortality[82,83], infection[84,85] and other complications[86,87]. Hyperglycaemia during critical illness can be attributed to pre-existing diabetes (both type 1 and type 2; 13%-20% of patients)[61,83], incidental/unrecognised diabetes (defined as HbA1c > 6.5% identified for the first time during acute illness; 5%-15%)[88-90] or stress hyperglycaemia (defined as a peak blood glucose concentration that, in health, would lead to a diagnosis of diabetes; 17%-50%)[91-94]. The underlying mechanisms of acute hyperglycaemia in the critically ill include increased insulin resistance[95] and relative insulin insufficiency[96]. Long-term consequences of stress hyperglycaemia include a higher rate of subsequent diabetes and its associated complications[97,98].

Exogenous insulin used to achieve glycaemic control can cause hypoglycaemia and increased glycaemic variability, both of which have an impact on mortality[61,99,100].

Gastroparesis in critically ill patients

In the critically ill, nutrition is most commonly delivered *via* the nasogastric route and success is, accordingly, dependant on intact gut function. Delayed GE is common, (50%-80%), as indicated by large gastric residual volumes (GRVs), and associated with early cessation of enteral nutrition, increased infection, increased length of stay and increased mortality[101-103]. Surprisingly, pre-existing type 2 diabetes does not appear to be a risk factor for delayed GE[104], suggesting that the delayed GE in critical illness is mechanistically unrelated. We have reported that the rate and extent of glucose absorption following intragastric administration is markedly reduced in about 1/3rd of intensive care unit patients[46] and is dependent on the rate of GE[105]. Thus, GE is a major determinant of postprandial glycaemia in this group[1,13] and may predispose to increased glycaemic variability[106]. Furthermore, delayed GE in patients treated with insulin may represent a risk factor for hypoglycaemia [37]. Likewise, acute hyperglycaemia has been associated with delayed GE in the critically ill[46]. Due to the interdependent relationships and extent of glycaemic variability noted in many studies there are likely to be multiple factors affecting this relationship in both directions. Thus, interventions aimed at overcoming delayed GE, for example the use of prokinetics, post-pyloric tubes and parenteral nutrition, may have as yet unidentified effects on glycaemia. Prokinetic therapy can improve critical illness gastroparesis and has been associated with better clinical outcomes[107], but its impact on glycaemic variability is uncertain[108-110].

Role of feed composition

The macronutrient composition of feed formulae is likely to have both direct and indirect effects on glycaemia, the latter by affecting the rate of GE. Energy dense and high lipid feed formulae are associated with slower GE (*i.e.*, emptying proceeds at a specific caloric rate (kcal/min) and is, accordingly prolonged) with no significant improvement in glycaemic control[111]. The large, multicentre TARGET trial, reported that the administration of a high density formula (additional calories from additional lipid and carbohydrate) resulted in both hyperglycaemia requiring higher insulin doses [112] and larger GRVs. The additional carbohydrate is likely to account for the higher blood glucose and the increased lipid could contribute to the slower GE. As these parameters are interrelated, it is impossible to determine from this study whether, and by how much, hyperglycaemia per se is causing the slowing of GE or vice versa. Rugeles *et al*[113] reported less hyperglycaemia with high-protein hypocaloric feeds. In another pilot RCT (FEED trial) comparing the effect of two protein doses (1.2 g/kg/day *vs* 0.75 g/kg/day) on muscle mass, no difference in feed intolerance (GRV > 300 mL) was evident[114]. In another pilot study investigating the feasibility of delivering higher protein doses (1.52 ± 0.52 *vs* 0.99 ± 0.27 g/kg/d), there was no difference in glycaemia and mean daily GRVs were less [115]. High protein feed formulae may, accordingly, potentially result in less GI intolerance and dysglycaemia, but this requires confirmation in larger studies.

GLP-1 based therapies in the management of glycaemia in critical illness

Insulin remains the most frequently used medication to treat hyperglycaemia in critically ill patients. Most other oral anti-antidiabetic medications are withheld in intensive care patients due to their unpredictable absorption and concerns about their impact on glycaemic variability and variable nutrition intake. Long-acting insulin is sometimes used in patients tolerating enteral nutrition for sustained glycaemic control during the recovery phase of illness due to the convenience of administration. However, in the acute phase of critical illness, short-acting, continuously infused, IV insulin is generally used. This carries the risks of increased glycaemic variability and hypoglycaemia, necessitating intensive monitoring. Thus, other medications that can normalise elevated blood glucose levels and reduce glycaemic variability and the risk of hyperglycaemia are being explored.

Gut-based antidiabetic therapies (*e.g.*, incretin hormones) may offer a safe yet effective alternative to insulin. Our group has published 'proof of concept' studies over the past decade in which we have demonstrated that exogenous GLP-1 infusion attenuates, but does not normalize, hyperglycaemia induced by enteral nutrition in critically ill patients with both type 1 diabetes[116] and stress hyperglycemia. The slowing of GE by GLP-1 appears to be a plausible contributory mechanism[105]. IV GLP-1 may also reduce glycaemic variability, although in this small study it did not appear to impact IV insulin requirements or the frequency of hypoglycaemia)[117]. This study was also limited by the dosing of the medication (FDA mandate limiting GLP-1 dose to 1.5 pmol/kg/min) and the magnitude of glucose lowering (desired glucose range of 4.44–6.11 mmol/L was achieved in only a minority of patients)[117]. The use of GLP-1RAs is of interest, mainly due to the low risk of hypoglycaemia, given the glucose-dependency of the insulinotropic effect and glucagon suppression in comparison to currently used IV insulin therapy. The impact of GLP1-RAs on glycaemic management, GE, nutrition delivery and medium and longer term clinical outcomes in critically ill patients is not known. A potential limitation related to their current SC, rather than IV, use and the lack of safety data in the critically ill. It should be appreciated that GLP-1RAs have cardiac and renal protective effects with

longer-term use which may be of relevance[118].

CONCLUSION

GE has an important and inter-dependent relationship with the acute glycaemic environment in health, diabetes, and critical illness, which is relevant to clinical practice. Abnormally delayed GE, or gastroparesis, is common in type 1 and type 2 diabetes, and in critical illness. Recent insights have led to a better understanding of the pathophysiology of diabetic gastroparesis, especially at the cellular level. Glucose-lowering medications such as GLP-1RAs that act primarily by slowing GE, are used widely today in the management of type 2 diabetes but their actions on GE under various glycaemic conditions are not known and their place in the management of dysglycaemia in critical illness remains uncertain. Advantages of reduced hypoglycaemia and glycaemic variability will need to be balanced against the potentially adverse impact of slowing of GE on nutrition delivery and the risk of aspiration. Further studies building on these insights and focusing on the interactions of medications affecting GE and glycaemic environment in hospitalised patients are required.

FOOTNOTES

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REVIEW

Early diabetic kidney disease: Focus on the glycocalyx

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Abstract

The incidence of diabetic kidney disease (DKD) is sharply increasing worldwide. Microalbuminuria is the primary clinical marker used to identify DKD, and its initiating step in diabetes is glomerular endothelial cell dysfunction, particularly glycocalyx impairment. The glycocalyx found on the surface of glomerular endothelial cells, is a dynamic hydrated layer structure composed of proteoglycans, glycoproteins, and some adsorbed soluble components. It reinforces the negative charge barrier, transduces the shear stress, and mediates the interaction of blood corpuscles and podocytes with endothelial cells. In the high-glucose environment of diabetes, excessive reactive oxygen species and proinflammatory cytokines can damage the endothelial glycocalyx (EG) both directly and indirectly, which induces the production of microalbuminuria. Further research is required to elucidate the role of the podocyte glycocalyx, which may, together with endothelial cells, form a line of defense against albumin filtration. Interestingly, recent research has confirmed that the negative charge barrier function of the glycocalyx found in the glomerular basement membrane and its repulsion effect on albumin is limited. Therefore, to improve the early diagnosis and treatment of DKD, the potential mechanisms of EG degradation must be analyzed and more responsive and controllable targets must be explored. The content of this review will provide insights for future research.

Key Words: Glycocalyx; Diabetic kidney disease; Endothelial cells; Reactive oxygen species; Microalbuminuria; Enzyme

Core Tip: In the diabetic microenvironment, various harmful factors, such as oxidative stress and inflammation, contribute to endothelial glycocalyx (EG) disruption through direct damage to the glycocalyx or indirect degradation due to the upregulation of related sheddases. Shedding one or more components after damage to the EG is an early sign of numerous pathological states, including diabetes. The loss of filtration barrier integrity can lead to microalbuminuria, which is predictive of diabetic kidney disease (DKD). Identifying and targeting the key molecules involved in glycocalyx damage thus represent current hot topics in DKD research.

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INTRODUCTION

Over the past 30 years, the number of people with diabetes mellitus has quadrupled globally, and approximately 1 in 11 adults currently have diabetes (mainly type 2)[1]. Moreover, most patients with diabetes also have complications, which seriously affect their quality of life and life expectancy. Diabetic complications are categorized as macrovascular (*e.g.*, cardiovascular disorders) and microvascular (*e.g.*, renal, retinal, and neurologic disease). In recent decades, cohort studies from high-income countries have shown that the relative risk of microvascular complications is at least 10 times higher in patients with diabetes than in patients without diabetes, while the relative risk of macrovascular complications is 2-4 times higher[2]. In developing countries, patients with diabetes have a higher risk of renal complications but a lower risk of coronary heart disease[3], which further reveals the increasing incidence of diabetic microvascular disease complications, especially diabetic kidney disease (DKD). However, the pathogenesis of DKD is incredibly complicated and remains poorly understood, and current treatments have limited efficacy. In the last ten years, DKD has replaced glomerulonephritis as the primary reason for chronic kidney disease in China[4] and it has also become the leading global cause of end-stage renal disease[5]. Understanding the pathogenesis of early DKD is, thus, of profound significance because it could aid in delaying, preventing, or reversing the progression of this disease and improving the prognosis of patients.

The glomerular filtration barrier (GFB) comprises three distinct layers: Endothelial cells, glomerular basement membrane (GBM), and podocytes. The pathological changes that occur with DKD include glomerular capillary hypertrophy, GBM thickening, podocyte foot process disappearance, and mesangial expansion. Microalbuminuria occurs prior to these changes and is denoted by a slight increase in the urinary excretion of albumin (20-200 μg/min in humans) prior to overt DKD, and is the first predictor that a patient has a high risk of developing DKD, with both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM)[6]. It is noted that renal tubules have powerful reabsorption, and a 50% increase in the filtration rate increases urinary albumin in the sub-microalbumin range[7]. Thus, to facilitate albumin flux increases that are sufficient to produce microalbuminuria, normal renal tubule reuptake requires structural alterations to the GFB[8]. Furthermore, endothelial dysfunction has been found to precede the onset of microalbuminuria[9]. The trigger for endothelial dysfunction is based on the permselectivity of GFB to molecules of different sizes and charges. The albumin filtration increase can be estimated, and it depends on the size or charge selectivity of the defect [10]. Studies have found that the occurrence of microalbuminuria is tied to charge selectivity in diabetic animal models and patients with T1DM and T2DM[10-12]. The lack of charge selectivity is observed earlier than the depletion of size selectivity, and the size selectivity defect only appears after the transition to the macroalbuminuria stage[12]. Consequently, the pre-emptive advantage of the charge selective defect suggests that the damage to the endothelial glycocalyx (EG) with a negative charge most likely represents the first step in the progression of microalbumin in patients with DKD. Thus, the structure and function of the EG, the mechanism of EG damage, and potential therapeutic strategies must be further explored to curb the rapid spread of DKD.

GLYCOCALYX STRUCTURAL AND FUNCTIONAL ALTERATIONS IN DKD

EG

Glomerular endothelial cells are highly differentiated with cytoplasmic decay zones dotted with many fenestrae, which are round transcellular pores at 60-80 nm in diameter[13]. The fenestrae were previously considered empty, which means that they are a weak barrier against albumin filtration[14].

Although fenestrated capillaries are much more permeable to water and small solutes than non-fenestrated capillaries, there is little albumin in the GBM and adjacent podocytes under physiological conditions[15,16]. Albumin is a polar protein with a total net charge ranging from -12 to -18 at physiological pH[17]. Studies using dextran with different charges found that polycationic DEAE dextran was cleared more at a certain molecular radius than neutral dextran, which was filtered more freely than negatively charged sulfate dextran[18]. These phenomena can only be explained by the negatively charged glycocalyx. In addition, the Starling hypothesis indicates the primary method of fluid exchange between plasma and tissue in most capillaries. On this basis, the revised Starling hypothesis states that at a steady state, colloidal osmotic pressure differences, which are resistant to hydrostatic pressure, exist across the EG rather than the entire vessel wall, effectively preventing albumin from leaving the vessel[19]. Observing the glycocalyx on the surface of the endothelial cells requires specific fixation and staining techniques. The immunofluorescence confocal technique is now widely used with lectin to fluorescently label glycocalyx components, which can be directly observed in the 200-400 nm thick glycocalyx covering the luminal surface of the glomerular endothelial cells in the fenestral and inter-fenestral domains. The EG is a complex layer on the glomerular endothelial cells composed of proteoglycans (PGs), glycoproteins, and glycolipids. It integrates components, such as plasma proteins, α-acid glycoproteins, antithrombin III, extracellular superoxide dismutase (SOD), lipase, growth factors, and chemokines, to form a looser layer known as the endothelial surface layer (ESL)[20]. The PGs of the EG consist of core proteins and glycosaminoglycan (GAG) side chains. To the best of our knowledge, the main core proteins are syndecans and glypicans. The GAGs include heparin sulfate (HS), hyaluronic acid (HA), chondroitin sulfate (CS), and keratan sulfate, which are all negatively charged due to their carboxyl and/or sulfate groups[21]. Short exposure to glucose levels > 15 mmol/L resulted in a 50% loss of the glycocalyx in healthy individuals[22]. In C57BL/6 mice, acute hyperglycemia increased EG permeability[23]. EG shedding increases the concentration of several types of EG in the blood or plasma, such as HA, HS, and syndecans. Thus, the plasma levels of these molecules can be regarded as a responsive indicator for EG degradation. Glycocalyx hydrolysis is closely related to sheddases, such as heparinase (HPSE), matrix metalloproteinase (MMP), hyaluronidase (HYAL), and neuraminidase (NEU)[24]. In patients with T1DM, the loss of approximately half of the body's glycocalyx was accompanied by elevated plasma HA and HYAL levels. More importantly, the glycocalyx volume was decreased in T1DM patients with microalbuminuria when compared with those without[25], and similar results were reported in patients with T2DM[26]. These findings suggest that the decrease in EG correlates strongly with microalbuminuria. Swärd and Rippe[27] proposed a more precise exposure time for hyperglycemia. Short-term (lasting minutes to hours) exposure to hyperglycemia produced microproteinuria *via* protein kinase Cα and downstream Rho-associated coiled-coil protein kinase pathways mediating F-actin cytoskeleton rearrangements, while long-term (lasting two weeks) exposure induced the permeability of glomerular endothelial cells to albumin associated with EG disruption. It should be noted that besides serving as a filter barrier, EG ensures vessel patency (through its antithrombotic and antiadhesive properties), transduces shear stress, regulates the vascular tone (by sensing fluid shear forces), and protects endothelial cells from oxidative stress (*via* combining free radical scavengers)[28].

Core proteins and MMPs: Syndecans and glypicans are the main core proteins in EG. Other core proteins, such as mimecans and perlecans, are soluble and secreted in both the EG and blood[29]. Syndecans are transmembrane proteins that mainly bind HS or CS chains[28,30]. There are six significant subtypes of syndecans[31], and syndecan-1 and 4 are particularly prominent in nephrons [32]. The former is connected to three HS chains[33], while the latter can carry 3-5 HS chains[32] and is most abundant in the syndecans family in human glomerular endothelial cells[34]. Glypicans are anchored to glycosylphosphatidylinositol and have four main isomers[30,31], of which glypican-1 binds almost exclusively to the HS chain[35], but close to the cell membrane, glypican-1 binds to 3-4 HS chains [33]. Heparan sulfate PGs (HSPGs), composed of syndecan and HS, are most abundant on the cell surface[36], followed by phosphatidyl inositol PGs, composed of glypicans linked to HS[37]. An essential function of the syndecan core proteins is to put the highly bioactive GAGs in the right place at the right time[38]. MMPs are a kind of zinc-reliant endopeptidase that are mainly synthesized by inflammatory cells, although MMPs can also be synthesized by endothelial cells and vascular smooth muscle cells when stimulated by macrophages[39]. MMP-2 and MMP-9 can be activated by MMP14 (also known as membrane type 1)[32]. MMP-2, MMP-9, and MMP-14 can cleave syndecans at different sites to produce various sizes of proteolytic fragments[40]. Typically, MMP-9 degrades syndecan-1 and MMP-2 cracks syndecan-4, allowing syndecans and HS to be released into the blood[39]. Diabetic conditions promote the overexpression of endothelial MMP-9[34], MMP-2[26], and urinary MMP-14 [41], and the activity of these MMPs is elevated in the kidneys of diabetic humans[41,42] and mice[43].

HS and HPSE: HS is the most common GAG in the glycocalyx and accounts for approximately 50%-90% of its amount[44]. It is comprised of 300 alternate N-acetyl-glucosamine a1 to 4 glucuronic acid b1 to 4 residues[45]. HS biosynthesis exists in the Golgi apparatus, and its characteristics include chain initiation, polymerization, and modification[46]. HS can be extensively modified, including N-deacetylation/N-sulfation of N-acetylglucosamine, isomerization of C5 glucuronic acid to iduronic acid,

and 2-O-, 3-O-, and 6-O-sulfation[45]. Different combinations of these modifications produce structurally diverse HS chains, which dictate the binding and modulation of specific proteins and regulate the activity of various biological molecules, such as cytokines and growth factors on the cell surface[45,47]. *In vitro* studies have found that high levels of glucose reduced HS synthesis and increased the monolayer albumin flux in glomerular endothelial cells, implying that the presence of HS in EG limits proteinuria[48]. The structure of the HS chain may be edited by HS modification enzymes, including HPSE, β (1-4)-endoglucuronidase, which clears HS at specific sites, and HS 6-O-endosulfatase, which explicitly removes 6-O-sulfonate[49]. HPSE is the most well characterized of these enzymes, and it is the sole mammalian endoglycosidase that cuts HS[50]. The nascent HPSE is inactive and requires activation by cathepsin L[46]. Active HPSE cleaves glycosidic bonds within the HS chain to yield HS fragments that are 5-7 kDa in size, and this cleavage requires the N-and 6-0-sulfated moieties to have specific sequences, such as the trisaccharide sequence GlcNS60S-α(1-4)-GlcA-β(1-4)-GlcNS6OS[51]. Intracellular HPSE has a variety of biological functions, including the regulation of cellular autophagy, communication, and survival. Conversely, extracellular HPSE is related to inflammation, vascular instability, and fibrosis and is a crucial contributor to renal damage in patients with DKD and glomerulonephritis[47]. The first study to reveal a role for HPSE in the development of proteinuria was performed on rats with puromycin aminoglycoside nephropathy, and it showed that HPSE overexpression was an essential contributing factor to HS loss in proteinuria[52]. In Zucker fatty rats proteinuria was associated with a significant glycocalyx reduction, and this was at least in part related to elevated HPSE levels[53]. However, specific HPSE inhibitors PI-88[54] or polyclonal anti-HPSE antibodies 226[55] reduced proteinuria levels and alleviated renal damage. At the same time, the overexpression of HPSE in transgenic over-expressing mice (HPSE-TG) resulted in early proteinuria and renal failure[56]. According to a previous study, the transcription factor early growth response 1 is responsible for activating the HPSE promoter under hyperglycemic conditions[50]. Compared with healthy volunteers, patients with DKD show increased urinary and renal HPSE activity[57]. Furthermore, HPSE was upregulated in response to a high-glucose environment and DKD mediators, such as advanced glycation end products (AGEs), in mouse DKD models[58] and renal-derived cell lines (endothelial cells, renal epithelial cells, and proximal tubular cells)[59-61]. In addition, Schmidt *et al*[62] proposed that there was a relationship between urinary HS and renal function. It was believed that urinary HS could predict the progression of renal dysfunction. However, the increase in permeability caused by glycocalyx injury was not enough to reduce the glomerular filtration rate (GFR), and the obstruction of the secondary capillary lumen, caused by leukocyte or platelet interactions with the endothelial cells, was the cause of GFR reduction.

HA and HYAL: HA is a non-protein-bound, non-sulfate, negatively charged GAG, a linear polysaccharide held together by the repeat units of D-glucuronic acid and N-acetyl-D-glucosamine by glycosidic bonds repeated thousands of times[39,63]. HA is synthesized from three isoforms of hyaluronic acid synthase (HAS). HAS-2 is the major synthetase of HA and is expressed in most cells and essential for life[63]. Van den Berg *et al*[63] found that the HAS-2 deletion in the endothelial cells of adult mice (selective inactivation of the HAS-2 gene in endothelial cells of adult mice carrying the floxed HAS-2 allele) substantially reduced the glycocalyx structure. Importantly, HA is a specific binding site for angiopoietin-1 (Ang-1) and a key regulator of endothelial cell quiescence and maintenance of endothelial barrier function. HA deficiency triggers Ang-1-Tie2 receptor signaling disorder, which is characterized by vascular instability, mesangial dissolution, telangiectasia, and proteinuria, and gradually progresses to glomerular capillary rarefication and glomerulosclerosis, which produces the human DKD phenotype. They observed endothelial HA in renal biopsies from patients with different severities of diabetes and found that endothelial HA in glomerular capillaries gradually disappeared with the formation of DKD lesions. In patients with acute hyperglycemia or T1DM, the EG volume decreased by 50%-80% and the serum HA concentrations increased by 30%-80%[20]. Degradation of the EG and increased serum HA concentrations were also observed in T2DM patients[26] and rodent models of T1DM[64]. This shedding of HA resulted in increased vascular permeability with albumin escape. Despite the prominent role of HA in EG, its method of binding to the cell membrane and integrating with EG is unknown. Although HA may bind to the cell surface receptor CD44, covalent bonding is not observed, and it may also attach to the extracellular part of HAS, interact with CS on syndecan-1, connect with EG through HA-binding proteins, or even independently assemble into a fibrous network[20]. Increased glycosylation of CD44 was reported to weaken its ability to bind HA in a high-glucose environment, thereby decreasing HA binding to EG[65]. The human body contains six kinds of HYAL to degrade HA. HYAL-1 and HYAL-2 are common in mammalian tissues and cooperate to complete HA degradation[66]. HYAL-2 is a glycosylphosphatidylinositol anchoring enzyme that attaches to the outside of the cell membrane[67] and is accountable for the extracellular degradation of high molecular weight HA into an intermediate fragment that is approximately 20 kDa[39]. The intermediate HA fragment is then endocytosed into the cell *via* endocytic vesicles and degraded into a small fragment by HYAL-1[68]. Interestingly, there are large differences between high and low molecular weight HA. The former can enhance endothelial barrier function, but the latter can damage endothelial cells in various ways. For instance, low molecular weight HA induces endothelial cell

inflammation through Toll-like receptors 2 and 4, stimulates the expression of vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1), leading to macrophage infiltration, cell inflammation, and injury, and it activates phagocytes to generate reactive oxygen species (ROS) in a size-dependent manner[39]. A study by Dane *et al*[69] found that 4 wk after injecting HYAL into C57BL/6 mice, the glomerular albumin permeability increased by 90% and the EG returned to integrity 4 wk after performing the injection. However, no significant proteinuria was observed during the experience, and this was possibly due to the protective effects of the normal apolipoprotein-E (apo-E) levels in these animals. Similar to the findings in apo-E absence mice, HYAL infusion results in EG disorder and proteinuria[70]. Several studies have also shown that the increase in HA concentration and HYAL activity, resulted in a thinning of the glycocalyx due to HA degradation, and this increased the transcapillary escape rate of albumin in mice with diabetes[71,72] and humans with T1DM[25] and T2DM[26]. Furthermore, Dogné *et al*[73] demonstrated that increasing the EG depth and maintaining the HA content during early DKD in HYAL-1-deficient mice contributed to preserving endothelial function and the functional barrier. Similarly, supplementation of HA analogs could compensate for glycocalyx loss. Thus, HA shedding could be utilized as a valuable observation for the pathogenesis of diabetic renal complications, and the presence of HA may prevent the emergence of early DKD.

Sialic acid and NEU: Sialic acid (SA), otherwise known as N-acetylneuraminic acid, is a constituent of cell membrane glycoproteins and glycolipids[74]. SA occurs at the glycocalyx surface and participates in signal recognition and the binding of sugars to proteins[39]. NEUs, tagged as sialidases, are a family of enzymes that regulate cell surface SA expression by removing SA from the glycocalyx[75]. Puerta-Guardo *et al*[76] found that nonstructural protein 1 induced NEUs expression, causing SA shedding and EG degradation. It could also activate cathepsin L in endothelial cells and affect HPSE activity[77]. What counts is that NEU could remove most of the glycocalyx and influenced the water, small solutes (as measured by transendothelial electrical resistance), and albumin fluxes, whereas HPSE (using HPSE III or recombinant HPSE-1), which removed HS GAGs alone, only had a remarkable impact on albumin filtration without changing water and small solute passages[78]. The results indicate that NEU is the most efficient enzyme with which to remove glycocalyx residues. Other studies have found that SA may directly regulate ESL permeability by steric hindrance and/or inducing secondary changes in ESL, such as the disruption of the albumin binding to EG[79]. Whether they really participate in the filtration barrier is currently unknown and this will require further research.

Cell adhesion molecules: Cell adhesion molecules include selectin, integrin, and the immunoglobulin superfamily[80]. Two major kinds of selectins are observed: P-selectin and E-selectin. P-selectin is produced and stored in the Weibel-Palade bodies found in endothelial cells and secreted in response to thrombin and histamine stimulation[81]. E-selectin is *de novo* synthesized in response to the stimulation of cytokines, such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and lipopolysaccharide[82]. Integrins are heterodimeric membrane proteins made up of noncovalently bound α and β subunits. The luminal membrane of endothelial cells expresses integrin $\alpha_v\beta_3$, which influences the interaction between platelets and endothelial cells[83]. The immunoglobulin superfamily glycoproteins include ICAM-1 and ICAM-2, VCAM-1, and platelet endothelial adhesion molecule-1, which promotes the adhesion of leukocytes and platelets to endothelial cells[84]. The Ib-IX-V complex, another well-defined glycoprotein consisting of four different proteins (Ibα, Ibβ, IX, and V), binds to the von Willebrand factor and P-selectin to accelerate hemostasis[85]. GAG chains cover adhesion molecules in the physiological state, which sterically prevents leukocytes or platelets from binding to the cell adhesion molecule receptors. However, EG degradation activates and exposes adhesion molecules, and this contributes to increased leukocyte adhesion and thrombosis[86].

Shear stress changes and proteinuria: The source of proteinuria in DKD may arise from damage to the glycocalyx caused by alterations in shear stress. Shear stress is the mechanical force exerted by blood flow on the vessel wall. It significantly influences the structure and function of endothelial cells. The specific location and composition of the EG determine its unique function as a mechanosensor. The GAGs that extend into the extracellular region, which may deform, transmit the shear stress of the perceived blood flow to the core protein components, triggering core protein displacement. The cytoplasmic domain of the core proteins is linked to signaling elements, such as G-protein receptors, including those associated with endothelial nitric oxide synthase (eNOS) formation and cytoskeletal elements, such as actin[87], and this regulates transcription in the nucleus[20]. Florian *et al*[87] identified HS-GAG as a mechanosensor for the NO response, which is involved in mechanosensing and mediates NO production under shear stress. The depletion of syndecan-1 or 4 altered the mechanosensing and cell alignment[88,89]. In addition, reports suggested that degradation of EG by NEU and HYAL reduced flow-induced NO production[90], indirectly confirming the indispensable role of HA and SA in mechanical transduction. Furthermore, the glycocalyx participates in the scattering of concentrations of the agonists, and flow changes affect agonists' distribution, thus transferring flow conditions to the cells. This results in variations in magnitude and the temporal and spatial distributions of the shear stress will modulate vascular tone and induce alterations in endothelial permeability and hydraulic conductivity, cytoskeletal structure, surface adhesion molecule expression, and gene expression[87].

The glycocalyx is undoubtedly present in podocytes and GBM, but there are differences in its composition and structure. The GBM glycocalyx was once considered the charge-selective barrier of the glomerular filtration layer. In recent years, an increasing number of studies have broken this traditional concept and suggested that the glycocalyx does not function as a major negative charge barrier in the GBM. The loss of its anion site does not lead to proteinuria. By comparison, the podocyte glycocalyx may, together with EG, constitute a "defensive line" that restricts albumin filtration.

Glycocalyx in the GBM

The primary components of the GBM include type IV collagens with α3, 4, and 5 chains, laminin β2, negatively charged GAGs, and core proteins, such as agrin, perlecan, nidogen, and collagen XVIII[24,91]. Laminin and collagen networks are the main determinants of the penetrative and selective barrier functions of the GBM[24]; however, their roles are beyond the scope of this article. Agrin is mostly produced by podocytes, has a molecular mass of 212 kDa, and carries at least two HS chains, and it constitutes the staple PGs of the GBM in all adult species studied[92]. Perlecan, with a molecular weight of 467 kDa, is mainly produced by glomerular endothelial cells and is attached to three HS side chains by the N-terminal domain I attachment sites. It occurs in the GBM during development but after this stage it is predominantly found in the mesangial matrix and Bowman's capsule, as is collagen XVIII[93,94]. The HS in the GBM consists of alternating glucosamine and D-glucuronic acid/L-aduronic acid residues, which are negatively charged due to the presence of multiple carboxyl and N-, 2-O-, 6-O-, and 3-O- sulfate groups[95]. Additionally, the carbohydrate side chain SA is involved in the formation of the negative charge for GBM[96]. Previous studies believed that GAGs in the GBM, including HS, could repel negative charges, including albumin, and prevent their filtration. For example, ferritin and bovine serum albumin filtration occurred by inculcating bacterial GAGs degrading enzymes to remove GAGs at the original site of the GBM[97,98]. Injection of the anti-HS antibody JM403 causes hematuria and albuminuria in rats[99]. In many renal diseases, such as DKD, lupus nephritis, minimal change disease, and membranous nephropathy, the content of HS in the GBM was reduced and inversely correlated with urinary protein excretion levels[51,100].

However, the primary negative charge-dependent barrier function of the HS in the GBM does not stand up to scrutiny. One key component of HS assembly is the *Ext1* gene product-the HS co-polymerase subunit. Using podocyte-specific *Ext1* knockout (PEXTKO) mice to stop the polymerization of HS secreted by podocytes, Chen *et al*[101] found that the glomerulus foot process disappeared and mild and non-significant proteinuria occurred. To verify the presence or absence of anion sites in the GBM, they used GBM-specific HS-GAGs monoclonal antibody and polyethyleneimine staining to show a significant and sustained reduction in glomerular capillary wall HS-GAGs. Nevertheless, it is important to note that the staining of the HS-GAGs in the glomeruli was not wholly eliminated as mesangial and endothelial cells could still assemble HS-GAGs. Hence, HSPG secreted by podocytes is not necessary to limit proteinuria, and other mechanisms may exist. In this study, it cannot be ignored that the HSPG secreted by podocytes appears to have the ability to control podocyte behavior. However, Harvey *et al*[102] reported that GBM-specific agrin knockout mice did not develop podocyte foot process effacement. This suggests that neither HS-GAGs on agrin nor the agrin core proteins are critical in mediating foot process morphology. In immortalized podocytes, the loss of EXT1 results in the absence of not only ECM-related HS-GAGs but also cell membrane-associated HS-GAGs. From a cell-matrix interaction perspective, the podocyte phenotype of PEXTKO may be due to the podocyte surface HSPG's inability to interact with HS-GAG-binding proteins such as laminin in the GBM[101]. Research investigating perlecan-HS and perlecan/agrin-HS dual mutated mice showed that anionic sites were significantly decreased within the GBM. However, the glomerular structures and renal functions were not altered overall and measurable proteinuria was not observed[103]. This indicates that the major role of HS in the GBM as a charge-selective barrier of capillaries can be ruled out. However, further research into the function of the HPSE and HS of the GBM in proteinuria production under pathological conditions, such as the Streptozotocin (STZ)-induced albuminuria in HPSE-TG mice or PEXTKO mice, is still required[51].

Podocyte glycocalyx

Several studies have been performed on the glycocalyx of podocytes. According to the different structures, podocytes can be separated into four areas: Apical membrane area of foot processes, cytoskeleton area, hiatus membrane between foot processes, and GBM junction area (bottom of foot processes of podocytes). The apical membrane region of the foot process is rich in SA and sulfate PGs, which provide the surface layer of the foot process with an anion charge barrier. The podocyte skeleton region, comprised of microtubules and filaments, maintains the typical morphology of podocytes and foot processes. The 25-60 nm hiatus between adjacent foot processes is connected by the slit diaphragm (SD), a specialized tight junction of proteins, including nephrin and podocin. This has been considered the central area for size selectivity in the filter barrier. However, Lawrence *et al*[104] recently stated that size-selective penetration into the lamina densa of the GBM and the podocyte glycocalyx, coupled with saturable tubular trapping, determines the macromolecules that enter the urine without direct size selection through the SD. Unlike the glycocalyx on the apical membrane region of the podocyte, the GBM junction area is covered by a unique glycocalyx[105]. It is presumed that the glycocalyx between

the podocytes and GBM could be responsible for a portion of the charge selectivity of the GFB[24]. Dystroglycan, a highly glycosylated protein, is mainly localized in the basolateral and apical membranes of the cell. It can act as a receptor for laminin and agrin in the GBM and maintain SD structures by charge repulsion[91]. Significantly, the major salivary protein of the podocyte glycocalyx is podocalyxin, and this is mainly expressed in the apical membrane area and is highly glycosylated by 20% hexose, 4.5% SA, and N-acetylglucosamine[106]. In the human minimal change disease model simulated by puromycin aminoglycosides, the podocalyxin SA content in podocytes decreased, and foot processes fused, suggesting that the foot process fusion in puromycin aminoglycoside nephropathy was related to the reduction in SA[107]. Likewise, perfusion of the isolated rat kidney with polycations (*e.g.*, protamine sulfate) to neutralize the polyanionic surface led to podocyte foot process retraction, SD displacement, and tight junction and gap junction formation between foot processes[108]. These phenomena considered that protamine sulfate neutralizes the negative charges of sulfate and SA residues on the PG membranes, which in turn altered podocyte morphology and intercellular connections through the attachment of ezrin protein and Na^+/H^+-exchanger regulatory factor 2 to the actin cytoskeleton, increasing albumin filtration through the podocyte[109]. In addition, it was also reported earlier that decreased podocyte-associated sulfate carbohydrates in DKD contribute to abnormally elevated urinary albumin excretion rates[110]. Thus, the loss of the podocyte glycocalyx charge and the secondary changes in podocyte morphology may have caused abnormal albuminuria.

However, the role of the podocyte glycocalyx in DKD is still in dispute. Garsen *et al*[111] used podocyte-specific endothelin receptor type A (ETRA or Ednra)/ETRB or Ednrb deficient (podETRKO) mice to induce diabetes. They found that diabetic wild-type (WT) mice displayed increased cortical HPSE mRNA, glomerular HPSE protein expression, and glomerular HPSE activity, whereas glomerular HS expression was decreased. The glycocalyx thickness of endothelial cells and podocytes was reduced by approximately 50%-60%, with significant proteinuria. In contrast, in diabetic podETRKO mice, HPSE and HS expression was normal, only the podocyte glycocalyx decreased by approximately 25%, and the proteinuria decreased significantly. The reduced podocyte glycocalyx thickness in the podETRKO mice appeared to be insufficient to produce albuminuria. Nevertheless, they did not rule out the possibility that proteinuria in diabetic WT mice required a combined reduction in the endothelial and podocyte glycocalyx. Furthermore, the critical role of growth factors in podocyte-endothelial crosstalk involves maintaining glycocalyx integrity, and this will be discussed in the following section.

MECHANISMS OF GLYCOCALYX DAMAGE

The specific mechanisms of glycocalyx damage in the early stages of DKD that were associated with oxidative stress, proinflammatory cytokines, growth factors, transcription factors, and other factors were reviewed. It is of note that all these mechanisms will require further refinement beyond existing reports in future studies.

Oxidative stress

ROS overproduction is vital for the pathogenesis of DKD. Specifically, the generation of ROS with DKD results from mitochondrial production, NAD(P)H oxidase, and xanthine oxidase (XO), among others [112]. The damage to the glycocalyx caused by ROS can be summarized as follows: (1) ROS can degrade HA, HS, and CS, which will directly destroy the glycocalyx. ROS cleave HS chains primarily from the glomerular EG, disrupting the EG *via* a direct mode of action without affecting the GAGs biosynthesis pathway[61]; (2) ROS can upregulate the expression of related sheddases to degrade the glycocalyx. For example, ROS activates MMPs and dissolves syndecan domains to induce glycocalyx proteolysis, which causes the glycocalyx to fall off[113]; and (3) ROS are capable of inactivating endogenous inhibitors of neutrophil elastase, and the neutrophil elastase then binds the HS chains of the syndecans, leading to their degradation[114].

In DKD research, ET and ET-related receptors have always been a research focus. Recently, the specific involvement of mitochondrial ROS in endothelial injury in early diabetic mice was well documented. Mitochondrial DNA damage in the glomerular endothelial cells of DKD-susceptible mice and DKD patients was associated with increased glomerular Ednra expression[115]. Higher plasma ET-1 levels were also observed in diabetic patients and DKD animal models[116,117]. Ebefors *et al*[118] reported that podocyte-derived ET-1 increased the expression of HPSE and HYAL in glomerular endothelial cells through Ednra, thereby mediating ESL loss. In mice, endothelial damage (including glycocalyx damage), proteinuria, podocyte loss, and glomerulosclerosis induced by diabetes were mitigated by mitochondrial ROS scavenging or a specific Ednra blockade. Therefore, Qi *et al*[115] proposed that the upregulation of endothelial Ednra and the activation of circulating ET-1 characterize DKD susceptibility in mice and humans. Combined with previous control studies by Garsen *et al*[111] that involved diabetic podETRKO mice and WT mice, ETRA/ETRB deficiency was found to protect the endothelial and podocyte glycocalyx from HPSE degradation and reduce the production of proteinuria. It can thus be concluded that the overactivation of the ET-1 signaling path in the endotheliocyte and/or podocytes is a detrimental factor associated with glycocalyx damage and proteinuria in DKD. AGEs are

also involved in mitochondrial ROS production under high-glucose conditions. AGEs can act on the receptor of AGEs (RAGE) on podocytes and activate the nuclear factor kappa-B (NF-κB) pathway, leading to increased HPSE synthesis[119].

NAD(P)H oxidase appears to be an essential mediator of ROS generation in glomerular endothelial cells. Human glomerular endothelial cells treated in a high-glucose environment showed increased ROS production, and this could be blocked entirely by NADPH oxidase inhibitors[120]. NAD(P)H oxidase 2 (NOX2) and NOX4 have substantial roles in glycocalyx injury related to DKD. In the early stage of diabetes in Akita mice, NAD(P)H oxidase was activated in endothelial cells, and the ROS level was increased. Excessive ROS activated the transcription of HPSE *via* the nuclear translocation of the E-26 transcription factor[121]. To further validate that NAD(P)H oxidase activation initiated and worsened DKD progression, Nagasu *et al*[122] bred endothelium-targeted Akita mice overexpressing NOX2 (NOX2-TG-Akit mice), which exhibited reduced ESL and had further increases in their capillary permeability. When NOX2-TG-Akit mice were treated with gp91TAT, a NOX2-specific inhibitor, at 6-8 wk of age, glomerular tomato lectin staining was restored and similar to that in the WT mice. Besides the NOX2 subunits, NOX4 expression was also increased in diabetic kidneys[123] and was interconnected with inflammation. The ROS produced by NOX4 increased the damage to the macromolecules and led to the generation of advanced oxidation protein products, advanced lipid oxidation end products, and AGEs[124]. In the glomerulus, AGEs acted through RAGE to stimulate the release of proinflammatory cytokines and the expression of DKD-related molecules, such as vascular endothelial growth factor (VEGF), connective tissue growth factor, transforming growth factor-β, insulin-like growth factor-I, platelet-derived growth factor, TNF, IL-1β, and IL-6[125,126]. Overall, endothelial injury is clearly related to increased endothelial ROS production by NAD(P)H oxidase, represents a critical step in the pathogenesis of DKD, and may be a potential therapeutic target for its onset.

XO is mainly expressed in the liver and intestine[127]. In STZ-induced diabetic rats, XO expression was increased in the liver and was taken up by glomerular endothelial cells *via* blood circulation, where it could then bind with sulfated GAGs on the endothelial surface[128-130]. There was no difference in xanthine oxidoreductase activity in liver tissues between the WT and Akita mice, but the Akita mice showed higher xanthine oxidoreductase activity in renal tissues. Renal XO produced excessive ROS in endothelial cells, which led to a disturbance of endothelial homeostasis, a reduction of the glycocalyx, and proteinuria. Topi, a non-purine selective XO inhibitor, could reduce albuminuria by mitigating endothelial damage induced by glomerular oxidative stress from XO activation[131]. It was thus inferred that ROS causing glycocalyx damage in DKD originates, at least in part, from the XO system.

Proinflammatory cytokines

Inflammation is viewed as a vital mechanism in the development and progression of diabetes mellitus, and it persists for a long period before the onset of DKD[132]. TNF-α is a proinflammatory cytokine that can directly destroy the glycocalyx[133] but also increase the permeability of endothelial cells by activating MMP-9, mediating the destruction of the EG caused by syndecan-4 and HS shedding[34]. The clinical use of a TNF inhibitor (enalapril) attenuated glycocalyx loss in an experimental endotoxin model [134]. High levels of glucose could cause the abnormal regulation of TNF-α mediators, producing microalbuminuria[135]. In patients with type 2 DKD, the increase in serum IL-1β preceded the increase in serum HS, suggesting that abnormal inflammasomes predate and may contribute to the impairment of EG[132]. Reine *et al*[136] showed that IL-1β, *via* MMP-9, induced syndecan-4 shedding in conditionally immortalized human glomerular endothelial cells in a dose-dependent manner. The NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome is one of the most comprehensively studied inflammasomes involved in the emergence and development of various inflammation-related diseases, and diabetes is no exception[137]. The NLRP3 inflammasome activates IL-1β and IL-18, which can both subsequently activate the intracellular signaling molecule MyD88 by binding to the cell surface receptor, and this activates the NF-κB signaling pathway. The activated NF-κB signaling pathway can increase the secretion of proinflammatory mediators, such as cytokines and chemokines, and ultimately destroy the EG[138]. The damage to the vascular EG exposes ICAM-1 and VCAM-1. Circulating leukocytes are, thus, more likely to adhere to the endothelial cells, further contributing to inflammation and endothelial dysfunction. Furthermore, fragments produced by glycocalyx degradation induce the polarization of T helper 1 cells, which subsequently induces the upregulation of CD44 and Toll-like receptors 2 and 4. This results in the adhesion and rolling of macrophages and monocytes, activation of the NF-κB pathway, and upregulation of the expression of HPSE, MMPs, HYAL, HAS, and NEU[39]. Diabetic inflammatory conditions and glycocalyx shedding cause a vicious cycle, and thus, the integrity of the glycocalyx must be protected under inflammatory conditions.

Monocyte chemoattractant protein-1 (MCP-1) is involved in the recruitment of monocytes, the migration of monocytes and macrophages, and the regulation of macrophage differentiation. Once chemokines are induced, chemical ligand gradients or chemokine gradients are formed for the directed migration of cells expressing appropriate chemokine receptors. Moreover, these gradients are formed along extracellular structures, such as the HS GAGs of the glycocalyx[139]. In patients with DKD, MCP-1 was increased in renal tissues and urine[140]. Even in the early period of DKD, macrophages can be identified in the glomeruli[141]. Infiltrating macrophages could secrete cathepsin L, activate HPSE, and

disrupt EG. The C-C chemokine receptor type 2 (CCR2) is an MCP-1 cognate receptor. The blockade of CCR2 with the small molecule CCX140-B was found to reduce proteinuria in patients with DKD[142]. An animal study by Boels *et al*[143] showed that the treatment of diabetic apo-E knockout mice with an MCP-1 inhibitor, the Spiegelmer emapticap pegol (NOX-E36), for 4 wk, resulted in the polarization of tissue macrophages to an anti-inflammatory phenotype, restoration of glomerular EG, and the reduction of albuminuria, despite the persistent loss of podocyte function. Meanwhile, in a double-blinded, randomized, multicenter pilot study, NOX-E36 was also observed to be safe and well tolerated and to have beneficial effects on the urinary albumin/creatinine ratio and hemoglobin A1c in patients with T2DM and albuminuria in five European countries[144]. The cellular mechanisms involved in EG degradation remain obscure. It has been reported that proinflammatory factors such as TNF-α activate mast cells to generate HYAL, HPSE, and MMP-9/2[39]. Mast cells can also activate adipose tissue cells to release HPSE, which can then degrade HS chains[145].

Growth factors

The crosstalk between glomerular endothelial cells and podocytes is a major event in the progression of DKD, in which growth factors play a pivotal role[146]. There are five VEGF variants in humans, VEGF-A, -B, -C, -D, and the placenta growth factor[147]. VEGF-A_{165}, a VEGF-A splice variant, is the most abundant isoform in the human body. It mainly forms and maintains endothelial fenestration through VEGF receptor 2 (VEGFR-2)[148]. VEGF-A_{165} was upregulated during the early stages of DKD in both humans[149] and in experimental models[150]. Moreover, VEGFR-2 was also upregulated in early DKD and associated with enhanced glomerular endothelial VEGF-A_{165}-VEGFR-2 signaling[151]. VEGF-A_{165} boosted the production of MMP-9, A disintegrin, and metalloproteinase domain 17, and increased the removal of sulfate GAGs from the glycocalyx[152,153]. In contrast, VEGF-A_{165}b protected the EG, as demonstrated in an early mouse model of T1DM. The application of human recombinant VEGF-A_{165}b restored glomerular EG thickness, possibly *via* delayed downstream signaling by VEGF-A_{165}b-induced VEGFR-2, thus indicating VEGFR-2/VEGFR-1 heterodimer formation[154]. Aside from VEGF-A_{165}b, VEGF-C can also antagonize VEGF-A/VEGFR-2 signaling and reduce macromolecular protein passage [78]. The VEGF-C treatment blocked the VEGF-A-induced increase in glomerular permeability *in vitro* and rescued the elevated albumin permeability in the glomeruli of type 2 diabetic mice with proteinuria. Glomerular albumin permeability was increased in mice when administered either acutely (30 min) or chronically (2 wk) with shedding enzymes, but VEGF-C blocked this effect while maintaining the EG depth and/or coverage[155]. Most importantly, VEGF-C could also induce HA and CS synthesis and significantly increased the expression of N-deacetylase/N-sulfotransferase-2, which is responsible for adding a sulfate group to GAGs to increase the negative charge of the glycocalyx[153]. Angiopoietins are another type of endothelial cell growth factor that interact with VEGF to regulate endothelial cell permeability[31]. The two paramount members of the Angiopoietins family are Ang-1 and Ang-2[156]. For normal endothelial function, the receptor Tie2 must interact with VEGFR-2[157]. In normal physiological conditions, the phosphorylation of the receptor is mainly induced by Ang-1. However, in diabetic pathological conditions, the balance between these two isoforms is disrupted, and Ang-2 prevails, which results in increased HPSE-dependent glycocalyx degradation[158]. Furthermore, Ang-2 increases VEGF-A expression, which in turn reinjures the glycocalyx by upregulating MMP-9[152]. It is safe to conclude that VEGF-A_{165}b, VEGF-C, and Ang-1 can inhibit increases in the glomerular VEGF-A_{165} signal, rebalance the related sheddase, restore the EG layer, and reduce proteinuria in patients with diabetes.

Others

Krüppel-like factor 2 (KLF2), an essential member of the KLFs, is highly expressed in vascular endothelial cells and participates in the regulation of vascular tone, anti-inflammation, anti-thrombosis, angiogenesis, and other essential processes that are required to maintain vascular homeostasis[159-161]. According to research, KLF2 expression was reduced in STZ-induced diabetic rats. Compared with diabetic WT mice, diabetic KLF2 knockout mice showed increased glomerular expression of VEGF-A, VEGFR-2, and Ang-2 and decreased expression of VEGFR-1, Tie2, and Ang-1, as well as decreased expression of the zonula occludens-1 (ZO-1), glycocalyx, and eNOS. These data suggest that KLF2 down-regulation may contribute to glomerular endothelial cell damage in early DKD. The potential gene regulated by KLF2, NOS-3, reportedly encodes eNOS. In diabetic kidneys, eNOS expression may be inhibited by high levels of glucose, and KLF2 is required as a compensatory mechanism to maintain its expression. However, the specific mechanism by which KLF2 reduces endothelial damage in a diabetic environment will require further investigation. KLF2 may attenuate DKD by activating anti-oxidative stress and anti-inflammatory pathways[161]. Long non-coding RNA H19 was obviously increased in diabetic glomeruli and high glucose-stimulated rat glomerular endothelial cells. Deficiency or silencing of the *H19* gene could significantly relieve endothelial structural damage in diabetic rats by upregulating the expression of ZO-1, occludin, syndecan-1, and endothelial cell activation markers sVCAM-1 and sICAM-1 *via* the Akt/eNOS signaling pathway[162]. The antiaging gene *Klotho* encodes a single-channel transmembrane protein expressed in the kidney[163]. Klotho protein expression was diminished in the kidneys of patients with early DKD, Akita mice, and diabetic models like STZ-induced or db/db mice[164-167]. Moreover, *Klotho* gene deficiency aggravated glomerular injury in

diabetic models[166]. To date, the molecular mechanisms underlying Klotho loss and its contributions to diabetic glomerular injury have not yet been confirmed. Oxidative stress or the extracellular signal-regulated kinase, NF-κB, induces low-density lipoprotein oxidation and may suppress Klotho expression in Akita mice[167]. Kadoya *et al*[167] used lectin staining to measure glomerular ESL and discovered that Akita mice had distinctly smaller areas of positive staining than WT mice while KLTG Akita mice (obtained by crossing *Klotho* transgenic mice with Akita mice) had decidedly restored areas of positive staining and reduced albuminuria. As Klotho induces the expression of manganese SOD (MnSOD), which is a major superoxide scavenger and is resistant to oxidative stress, it was hypothesized that Klotho protects against glycocalyx damage by inducing MnSOD. Therefore, the recombinant Klotho protein may be a new target for future DKD treatments.

THERAPIES TARGETING GLYCOCALYX DAMAGE

At present, two targeted treatment strategies are available for glycocalyx damage: Replace the lost glycocalyx components directly and weaken or enhance the specific targets of the glycocalyx damage process to prevent further damage.

Glycocalyx replacement therapy

Attempts to supplement charge loss *via* GAGs have focused on sulodexide, a compound of small molecular mass GAGs (80% HS and 20% CS), which has been used to treat microvascular complications in patients with diabetes[168]. Initially, a few small studies were conducted which demonstrated its effectiveness in DKD patients with microalbuminuria[26,169]. Subsequently, however, two more extensive randomized, double-blinded, placebo-controlled studies were conducted, and they confirmed that treatment with sulodexide did not decrease proteinuria[168,170]. It is important to emphasize that many researchers believed that the role of sulodexide was underestimated in these later studies[171]. Furthermore, research is also required to determine whether sulodexide is absorbed through the gastrointestinal tract[31]. Using a transplantation-induced ischemia/reperfusion model, Jacob *et al*[172] discovered that albumin supplementation reduced glycocalyx shedding and leukocyte adhesion to the endothelial cells.

Glycocalyx degradation-blocking therapy

In addition to the HPSE antibodies or specific HPSE inhibitors that could prevent the degradation of GAGs, heparin (analogs) was also found to have a protective effect on DKD because it effectively inhibited HPSE activity. If heparin components with the maximum HPSE inhibitory effect and minimum anticoagulant activity are selected, then these heparin derivatives could function as inhibitors to protect the glycocalyx. Other potential targets may be the transcription, transport, and processing levels of HPSE[51]. A previous study showed that the steroid hormone vitamin D can reduce the expression of HPSE in damaged cells, both *in vivo* and *in vitro*, and its mechanism may be to directly bind to the HPSE promoter through its receptor, affecting the activity of the HPSE promoter[173]. In addition, RAAS blockers like angiotensin-converting enzyme inhibitors could inhibit HPSE activity [174]. Piperazine ferulate has been widely used in the treatment of various kidney diseases. It was recently reported that piperazine ferulate downregulates the expression of HPSE-1 and increases the expression of syndecan-1 by regulating the expression of AMP-activated protein kinase (AMPK), thereby reducing the degradation of the glomerular glycocalyx and alleviating the damage to the glomerular endothelial cell filtration barrier that was induced by high levels of glucose[175]. Manipulating the glycocalyx by inhibiting MMPs provides an attractive therapeutic target for DKD. MMP inhibitor therapy has become a reality in clinical settings, as, for example, tetracycline, an antibiotic agent, can inhibit MMPs at subantibiotic doses[176]. The development of more specific MMP inhibitors is expected to reduce some of the adverse reactions associated with the broad-spectrum MMP inhibitors currently involved in clinical trials[32].

Enhanced oxidative stress damages the glycocalyx in DKD, both directly and indirectly. The selection of targeted antioxidants is thus also essential. AdipoRon is an oral, synthetic adiponectin receptor agonist that activates the AMPK/peroxisome proliferation-activated receptor-α pathway, reducing high glucose-induced oxidative stress and apoptosis in endothelial cells and thus improves endothelial dysfunction[177]. The RAAS blocker telmisartan reduced proteinuria[178] and NAD(P)H-dependent oxidase activity[179] in T2DM patients. Pyrazolopyridine compounds, GKT136901 and GKT137831, were dual inhibitors of the NOX1 and NOX4 subtypes that reduced ROS formation in db/db mice[180]. Recent studies have found that Cyclocarya paliurus triterpenoids mitigate oxidative stress in endothelial cells through the ROCK pathway, reduce VCAM-1 and ICAM-1 levels, block glycocalyx damage, and ultimately improve renal endothelial function[181]. Most importantly, the Ednra receptor antagonist could reduce glomerular vasodilation, promote the binding of ET-1 to Ednrb, enhance NO synthesis, decrease the production of ROS, reduce glycocalyx damage, and change the glomerular permeability of albumin[182,183]. The first use of Bosentan was found to have little effect on reducing proteinuria in recent studies, while the use of Avosentan was also discontinued early due to the high incidence of

heart failure[184]. Although Endra has been reported to cause sodium retention (Ednra blocking reduces the constriction of efferent arterioles and hyperfiltration), most studies indicate that fluid retention results from Ednrb blocking because Ednrb activation in the renal collecting ducts promotes sodium and water excretion through sodium channels[185]. Based on pharmacological actions, it is believed that the selectivity of Bosentan (Ednra: Ednrb block = 20:1) and Avosentan (Ednra: Ednrb block = 50-300:1) for Ednra is reduced at high doses, resulting in sodium and fluid retention due to the Ednrb blockade[184]. If so, low-dose and highly selective ET receptor antagonists may be the way forward to improve the effective clinical use of this class of drugs. Atrasentan (Ednra: Ednrb block = 1200:1) is a selective receptor blocker[185]. Boels *et al*[183] found that Atrasentan therapy restored EG, reduced glomerular HPSE expression, increased the renal NO concentration, and significantly altered the glomerular macrophage M1 and M2 balance, eventually reducing the urinary protein creatinine ratio in diabetic apo-E deficient mice. This result was also verified *in vitro* in the co-culture of endothelial cells and pericytes exposed to laminar flow. Nevertheless, even with highly selective antagonists, increasing the dose may lead to fluid retention and heart failure. It underscores the necessity of drug combinations so that the benefits from Atrasentan treatments for nephropathy can be achieved while also reducing the incidence of adverse cardiovascular events. Ultimately, 0.75 mg/d Atrasentan as an adjunct to RAAS inhibition was identified as the optimal dose for renal protection, as this could minimize proteinuria but also had the lowest indicator for salt retention in patients with T2DM and DKD[186]. Another approach to avoiding heart failure is to use a combination of Ednra inhibitors with sodium-glucose cotransporter 2 (SGLT2) inhibitors. The ZENITH trial tested this hypothesis by randomizing chronic kidney disease patients with and without T2DM to receive Zibotentan in combination with the SGLT2 inhibitor dagliazine. The trial results are expected to be available in 2023[187]. The Ednra inhibitors are thus a welcome pharmacological addition that could help to further reduce the risk of renal outcomes in patients already treated with RAAS and SGLT2 inhibitors[188]. Moreover, the hypoglycemic agents SGLT2 inhibitors had beneficial effects on the endothelium, primarily through their anti-inflammatory and antioxidant effects[189]. The glucagon-like peptide-1 receptor agonist can lower the harmful effects of oxidative stress and inflammation in endothelial cell mitochondria by activating glucagon-like peptide-1 receptor[190].

Inflammation and oxidative stress are always inextricably intertwined. Inhibiting the NLRP3 inflammasome and IL-1β could reduce mitochondrial ROS production[191], and some NLRP3 inhibitory molecules, for example, MCC950, CY-09, OLT1177, and FT011, have been developed for *in vitro* and animal experiments[192-195]. Pentoxifylline (PTF), a methylxanthine-derived phosphodiesterase inhibitor, had powerful antioxidant properties when used alone[196] or in combination with angiotensin-converting enzyme inhibitor in small studies of DKD[197]. A meta-analysis reported that pentoxifyllines had a significant antiproteinuric effect in all patients with DKD, which might be attributed to a decrease in pro-inflammatory cytokines[198]. The transforming growth factor-β inhibitor pirfenidone[199] has also been found to improve oxidative stress in chronic hyperglycemic renal lesions in rats. However, the studies on this medicine are in their preliminary stages and further evaluations are required[200].

CONCLUSION

Approximately half of all patients with DKD may eventually develop end-stage renal disease and face dialysis treatment, creating serious health and economic burdens for countries, societies, and individuals. It is thus imperative that methods are developed to delay, prevent, or reverse DKD progression at an early stage. Microalbuminuria is the best predictor of high DKD risk. Endothelial dysfunction with glycocalyx damage has been identified as the first step in developing microalbuminuria in early DKD. The EG, or ESL, is a complex dynamic hydrated structure that is integral to the formation of the glomerular negative charge barrier. Under normal physiological conditions, the degradation and remodeling of the EG can maintain a balance that effectively prevents albumin filtration. However, in the diabetic microenvironment, excessive oxidative stress, inflammation, and other harmful factors combined with the presence of related degrading enzymes promote the increased shedding of glycocalyx components and homeostasis imbalance, leading to endothelial dysfunction and eventual proteinuria. In addition, the interaction between podocytes and endothelial cells and the joint shedding of the EG and podocyte glycocalyx are also one of the important causes of proteinuria. Therefore, targeting the key molecules in the glycocalyx damage mechanism to prevent the continuous loss of the glycocalyx or replace the lost glycocalyx components is thus a promising therapeutic strategy. Furthermore, this strategy also highlights the importance precision medicine will have in the future (Figure 1).

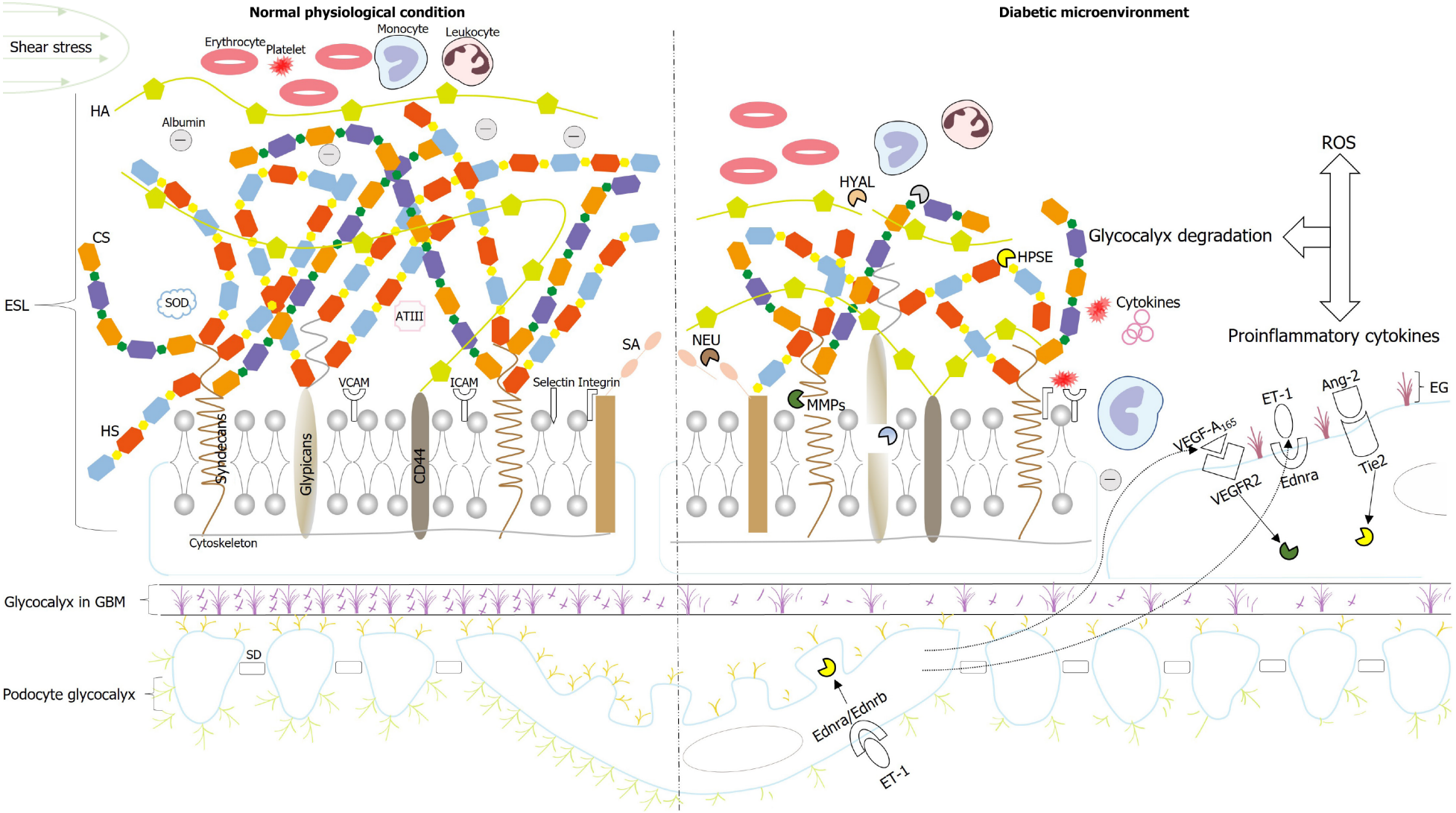

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Figure 1 The glycocalyx in the physiological state and the diabetic microenvironment. Under normal physiological conditions, endothelial glycocalyx shedding and recovery are in a state of equilibrium, which can form an albumin exclusion barrier on the endothelial surface. However, in the diabetic microenvironment, inflammation, oxidative stress, and other harmful factors can not only directly destroy the glycocalyx but also hydrolyze the glycocalyx by activating the related sheddases, such as heparinase (HPSE), hyaluronidase, matrix metalloproteinases (MMPs), and neuraminidase, resulting in the shedding of a large number of glycocalyx components, leukocyte and platelet adhesion, macrophage infiltration, and

microalbuminuria. In addition, the interaction between podocytes and endothelial cells plays a vital role in glycocalyx degradation. For example, the production of vascular endothelial growth factor A_{165} (VEGF-A_{165}) by podocytes acts on VEGF receptor 2 (VEGER2) in endothelial cells to induce the production of MMPs. Angiopoietin-2 (Ang-2) acts on Tie2 to increase the expression of HPSE, and Ang-2 also upregulates VEGF-A_{165} to degrade the glycocalyx further. In addition, there is an interaction between Tie2 and VEGER2. Endothelin-1 acts on endothelial cells Ednra to produce reactive oxygen species; it also acts on Ednra/Ednrb in podocytes to induce the production of HPSE to degrade the glycocalyx. HA: Hyaluronic acid; CS: Chondroitin sulfate; HS: Heparin sulfate; SA: Sialic acids; HPSE: Heparinase; MMPs: Matrix metalloproteinases; HYAL: Hyaluronidase; NEU: Neuraminidase; ROS: Reactive oxygen species; VEGF-A_{165}: Vascular endothelial growth factor A_{165}; VEGFR-2: Vascular endothelial growth factor receptor 2; ET-1: Endothelin-1; Ednra: Endothelin receptor type A; Ednrb: Endothelin receptor type B; Ang-2: Angiopoietin-2; SOD: Superoxide dismutase; AT III: Antithrombin III; ICAM: Intercellular adhesion molecule; VCAM: Vascular cell adhesion molecule; EG: Endothelial glycocalyx; GBM: Glomerular basement membrane; ESL: Endothelial surface layer; SD: Slit diaphragm.

FOOTNOTES

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REVIEW

Diabetes mellitus type 2 as an underlying, comorbid or consequent state of mental disorders

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Abstract

Somatic disturbances that occur in parallel with psychiatric diseases are a major challenge in clinical practice. Various factors contribute to the development of mental and somatic disorders. Type 2 diabetes mellitus (T2DM) is a significant health burden worldwide, and the prevalence of diabetes in adults is increasing. The comorbidity of diabetes and mental disorders is very common. By sharing a bidirectional link, both T2DM and mental disorders influence each other in various manners, but the exact mechanisms underlying this link are not yet elucidated. The potential mechanisms of both mental disorders and T2DM are related to immune and inflammatory system dysfunction, oxidative stress, endothelial dysfunction, and metabolic disturbances. Moreover, diabetes is also a risk factor for cognitive dysfunction that can range from subtle diabetes-associated cognitive decline to pre-dementia and dementia. A complex relationship between the gut and the brain also represents a new therapeutic approach since gut-brain signalling pathways regulate food intake and hepatic glucose production. The aim of this minireview is to summarize and present the latest data on mutual pathogenic pathways in these disorders, emphasizing their complexity and interweaving. We also focused on the cognitive performances and changes in neurodegenerative disorders. The importance of implementing integrated approaches in treating both of these states is highlighted, along with

the need for individual therapeutic strategies.

Key Words: Diabetes mellitus type 2; Mental disorders; Neuroinflammation; Neurodegeneration; Cognition

Core Tip: Mental disorders and type 2 diabetes mellitus (T2DM) are common, chronic, and frequently comorbid diseases that contribute significantly to global disability and mortality. Substantial evidence on the association between mental disorders and T2DM has been gathered over the past decade. In this review, we presented the latest cellular and molecular mechanisms of the shared pathways of T2DM and mental disorders, including neuroendocrine alterations and inflammation, immune response, oxidative stress, gut dysbiosis and gut-brain axis dysregulation, along with the hypothalamic-pituitary-adrenal axis dysregulation. The bidirectional link between mental disorders and T2DM underlines the importance of treating these disorders together rather than separately.

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INTRODUCTION

In the era of creating a concept of precision psychiatry[1], it is of utmost importance to acknowledge somatic disturbances that co-occur in mental disorders. Anamnesis vitae does not begin at the very moment of birth, yet it needs to include intrauterine development. Many factors can and do contribute to the future development of mental and somatic disorders. The interrelation of diabetes mellitus (DM) and mental disorders has fascinated both endocrinologists and psychiatrists for years. By sharing a bidirectional association, both DM and mental disorders influence each other in various manners, but the exact mechanisms underlying this link are not yet clear, and there are many questions that need to be addressed. The unique immunometabolic disturbances deserve special discussion because they could be associated with specific mental disorders later in life[2]. In this context, it is important to consider developmental programming or alterations of the intrauterine environment that induce compensatory responses and may persist in later life. Maternal diabetes during pregnancy could lead to neurodevelopmental outcomes, autism spectrum disorder, attention-deficit/hyperactivity disorder, and intellectual disabilities in the offspring, with increased risk for autism spectrum disorder and attention-deficit/hyperactivity disorder in pre-existing forms of diabetes, type 1 DM (T1DM) and type 2 DM (T2DM), but not with significance in gestational DM (GDM). For intellectual disorders, a two-fold increased risk was observed after exposure to T2DM compared to T1DM and GDM[3].

Synergistic effects of various factors could explain the multifactorial etiopathogenesis of mental disorders. T2DM could be seen in conjunction with different mental disorders. It could precede the onset of depression or could follow depressive symptomatology[4]. Anxiety overlaps diabetes microneuropathy[5], while eating disorders are accompanied by metabolic disturbances[6]. As we already discussed, intrauterine programming, lifestyle habits, or antipsychotic treatment could all contribute to diabetes onset in patients with schizophrenia[7]. Considering the worldwide burden of dementia, targeting a healthy lifestyle could prevent cognitive decline and preserve cognitive functions [8,9]. Recently, Dyer *et al*[10] have explored the precise timing and cascade of inflammatory mechanisms that convert physiological cognitive decline into dementia. A complex relationship between the gut and the brain also opens new therapeutic avenues, as gut-brain signalling pathways regulate food intake and hepatic glucose production. All these data have occupied our attention to explore the importance of T2DM in neuroinflammation and neurodegeneration. In this review, we aimed to enlighten the new concepts of T2DM etiopathogenesis that could contribute to mental disturbances and mental disorders symptomatology.

DM - THE BASICS

DM is defined as a complex and heterogeneous disease with a common state of hyperglycemia (Table 1). The American Diabetes Association considers T1DM as autoimmune β-cell destruction with absolute insulin deficiency and progressive loss of β-cells. This process is mediated by activated helper T

Table 1 Pathophysiology of various types of diabetes mellitus

Type of diabetes mellitus	Pathophysiology
Type 1 diabetes mellitus	Autoimmune β-cell destruction
Type 2 diabetes mellitus	Insulin resistance (liver, muscle, adipose tissue)
	Disorder of insulin secretion and β-cells breakdown
	Immune dysregulation and metainflammation
	Disorder of incretin production (glucagon-like peptide-1)
	Hyperglucagonemia
	Gut dysbiosis
	Increased glucose apsorption in stomach
	Kidney adaptation with increased glucose reabsorption and gluconeogenesis
	Decreased dopamine and increased sympathetic tone in brain
Type 3 diabetes mellitus concept	Impaired insulin and insulin-like growth factor-1 signaling
Gestational diabetes mellitus	Pregnancy induced glucose intolerance

lymphocytes which trigger effector cells of the immune system to destroy healthy β-cells. Simultaneously, a disruption of regulatory cells with a predominance of pro-inflammatory phenotypes occurs[11,12]. A hallmark of T2DM is significant insulin resistance and chronically increased β-cells engagement. The pathogenesis of this type of diabetes is multifactorial and has been investigated through the effects of various β-cell molecules[13-17].

GDM is defined as hyperglycemia occurring during pregnancy and registered during the second or third trimester. Although in 80% of cases, the main cause is marked insulin resistance caused by hormonal imbalance, the other 20% of cases are autoimmune in origin or other types caused by various factors that, even if they occur independently, can lead to the onset of the disease. These factors include genetic mutation, diseases of the exocrine pancreas, and drug- or chemical-induced diabetes[11].

ETIOPATHOGENESIS OF DM TYPE 2 - MODERN CONCEPTS

According to the World Health Organization, DM is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads to the development of chronic complications over time [18]. T2DM is one of the most common metabolic disorders worldwide, and it is estimated that the number of patients will increase significantly in the coming decades. Current analyses indicate the dominant representation of patients with T2DM (90%-95%) considering all patients with diabetes[11]. Patients with T2DM are mostly obese or have a higher body fat percentage, distributed predominantly in the central body region. At the same time, they have a 15% increased risk of all-cause mortality compared with people without diabetes[19]. The pathogenesis of T2DM is multifactorial and represents a combination of several simultaneous factors such as insulin resistance and β-cells deterioration, intestinal dysbiosis, and the presence of meta-inflammation (Table 1). The organs involved in T2DM development include the pancreas (β-cells and α-cells), liver, skeletal muscle, brain, kidney, small intestine, and adipose tissue[20,21].

Obesity is strongly associated with energy imbalance, characterized by increased food intake and decreased catabolism, and is associated with a state of chronic, low-grade inflammation, particularly in white adipose tissue[22]. Namely, as a result of long-term stimulation, adipocyte hypertrophy leads to the development of insulin resistance and reduced insulin-responsive glucose uptake in peripheral tissues[23]. Over time, the hypertrophy of adipocytes leads to their apoptosis. Apoptosis of adipocytes facilitates the accumulation of macrophages into adipose tissue, their differentiation toward the M1 phenotype, and subsequent production of proinflammatory cytokines[24].

Insulin resistance occurring in the liver unblocks glucose production in hepatocytes. This phenomenon is accompanied by additional glucogenesis in the fed state and even postprandially, which further leads to additional hyperglycemia[25]. All of the above-mentioned changes and the predominance of the pro-inflammatory response in the fat tissue and liver result in the reduced effect of insulin on peripheral tissues, compensatory hyperinsulinemia, and cause the burden of β-cells. Because of the long-term increase in insulin secretion, the accumulation of amylin takes a significant place in the decay of β-cells. This process is especially pronounced during the early phase of T2DM[26]. The enhanced function of β-cells, their deterioration, and the loss of compensatory hyperinsulinemia result in severe hyperglycaemia[27].

Another important aspect is the role of adipose tissue. Adipose tissue represents an important endocrine organ that regulates metabolism and behaviour through the production of adipokines. Among them, leptin, which is mainly produced in adipocytes, has a powerful influence on eating behaviour. Leptin-gene expression is extremely sensitive to acute energy balance, regardless of the long-term energy balance[28]. Short-term fasting decreases leptin messenger ribonucleic acid (mRNA) levels and plasma concentrations, whereas refeeding quickly restores its mRNA levels[29]. These changes suggest that leptin protects fat reserves against weight loss[30]. Leptin's access to key neurons in the central nervous system is of critical importance for its action. In obese people, the effect of leptin is weaker or absent[31], suggesting the disruption of its regulatory functions. Regarding the immunological functions of leptin, it has been shown that $CD4^+$ helper T cells cannot differentiate in the direction of T regulatory cells in states of elevated leptin[32]. In T2DM the main determinants of leptin levels are insulin secretion and the degree of insulin resistance[33].

Glucagon-like peptide-1 (GLP-1) is a hormone that regulates islet function, satiety, and gut motility with reduced secretion in patients with T2DM. McLean *et al*[34] have recently discussed new insights and refined their previous understanding of the GLP-1 function. In addition to the significant effects of GLP-1 on increased insulin production and reduced glucagon production, activation of GLP-1 receptors exerts hypophagic effects in the ventral hippocampus[35]. Numerous studies over the past decade have provided a deeper understanding of GLP-1 action in the brain. The direct link between gut secretion and the brain's GLP-1 system has not been found. GLP-1 receptor agonists exert their appetite-suppressing effects on cells in the circumventricular organs which transmit the signal to deeper brain structures[34].

During the last decade, it has been shown that the disturbance of intestinal flora, known as dysbiosis, occupies a significant place in the pathogenesis of T2DM. Dysbiosis represents an imbalance of commensal and pathogenic bacteria in the intestines and the production of microbial antigens and metabolites[36]. The occurrence of dysbiosis is accompanied by a disturbance of peripheral immune tolerance in the intestines with a predominance of dysregulated T-cell subpopulations[37]. The state of dysbiosis is accompanied by a disruption of the permeability of the intestinal epithelial barrier with the occurrence of hyperpermeability, also known as a leaky gut syndrome (LGS). LGS is defined as a condition in which intestinal endothelial cells allow microorganisms, their toxins, and antigens to "leak" into the bloodstream above the physiological values, consequently causing systemic reactions[38]. Dysbiosis is also accompanied by intestine inflammation[39]. The intestinal tract may develop an inflammatory response characterized by increased expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β), and IL-6 that leads to the development of insulin resistance[40]. In addition to dietary factors, pro-inflammatory cytokines also promote the formation of LGS. Interferon-gamma increases intestinal permeability by redistributing tight junction proteins and restructuring the cell cytoskeleton. TNF-α increases intestinal permeability by inducing apoptosis of endothelial cells[41]. On the other hand, IL-6 enhances intestinal permeability by altering the expression of molecules that play a major role in forming tight junction pores[42]. Alterations in transepithelial transport pathways may induce further translocations of harmful factors because of this vicious circle[43].

It is obvious that T2DM is associated with immune system dysfunction[44]. While T2DM can facilitate immune system activity in some tissues, it also negatively affects the immune response, which is confirmed by the higher incidence of unsuccessful vaccinations and complications of infections[45-47]. It appears that hyperglycaemia and pathologies in obesity, insulin resistance, and inflammation have a strong impact on the immunity of the host[48-50]. Various mechanisms have been proposed to be responsible for this phenomenon. Hyperglycaemia directly disturbs endoplasmic reticulum function, thus facilitating the accumulation of misfolded proteins in the lumen and promoting endoplasmic reticulum stress, which in turn modulates the function of immunocompetent cells[50]. Second, reactive oxidative species, which are abundant in the sera of patients with diabetes, alter innate immune cells activity through the diminished expression of activating receptors[51]. Taking everything into account, both innate and adaptive immune responses are altered in patients with T2DM and are not capable to provide adequate and effective protection against invading pathogens[45]. The logical outcome is a constant and permanent chronic inflammatory reaction in the immune response to pathogens and the resulting constant production of pro-inflammatory cytokines in amounts insufficient to initiate a strong immune response and elimination of pathogens, but still sufficient to induce many consequences in diabetic subjects.

T2DM AND COGNITION

Cognitive impairment and dementia are frequently accompanying and complicating T1 and T2DM[52]. 1.25-1.9-fold higher risk is established for cognitive dysfunction in diabetes[53]. There is increasing evidence that diabetes predisposes to cognitive decline leading to dementia[54,55], with a stronger link confirmed between dementia in T2DM than in T1DM. The risk for dementia progress increases with the aging of patients with diabetes, with a 50% higher risk in patients aged 75 years and over than in

patients aged 65-75 years[56]. Diabetes-associated decrements in their mildest stage can occur in all age groups, from young adults and even adolescents with T2DM[57] to the oldest patients[58]. A meta-analysis revealed that the domains of the speed of processing information, attention, concentration, executive functioning, and working memory were mainly influenced in diabetes compared to non-diabetic people[59].

The risk of diabetes-related cognitive decline was significantly increased in more severe clinical presentation and longer duration of T2DM[60,61]. Although the severity of diabetes is a risk factor for developing dementia[62], individuals without diabetes who have higher average glucose levels were also found to be at significant risk for dementia[63]. Diabetes does not act alone, but rather within a broader cluster of cardiometabolic disorders. Cognitive decline was associated with elevated blood sugar levels, a longer duration of diabetes, comorbid hypertension, and a history of a cerebrovascular event or myocardial infarction[64]. The impact of diabetes on the prodromal phase of dementia was demonstrated in the cohort of older adults and showed that poorly controlled diabetes increased the risk and progression of cognitive impairment, which was exacerbated by comorbid heart disease and mediated by systemic inflammation[65]. Hyperglycemia was observed as the main contributor to cognitive decline in metabolic syndrome[66,67]. Numerous epidemiological studies have identified diabetes and obesity measured in later life as risk factors for cognitive impairment[68]. Other comorbidities associated with aging and diabetes also add to the burden of cognitive impairment. Depression has been associated with a greater decline in cognitive function in patients with T2DM[69].

The exact pathogenic mechanisms underlying cognitive impairment in T2DM are not fully understood and are undoubtedly complicated, with numerous interacting factors (Figure 1). The cognitive impairments in diabetic encephalopathy have been associated with structural changes[70] and brain atrophy[71]. Cortical, subcortical, and hippocampal atrophy, particularly in the dentate gyrus, has been detected in T2DM patients by brain magnetic resonance imaging[71-73]. Various endocrinological, metabolic, and vascular abnormalities are DM-related and may precipitate the worsening of cognitive abilities.

Insulin could have a significant role in cognitive processing through the cerebrocortical activity of insulin receptors. They are allocated extensively in the hippocampus, entorhinal cortex, and frontal lobes, localities of the brain whose functions are involved in memory, attention, and executive functioning[74]. Variabilities in signalling pathways of insulin, phosphorylation of insulin receptor substrate 1, and altered signalling of insulin-like growth factor-1 were considered as main contributors to cognitive dysfunction pathogenesis[75,76].

Overexpression of proinflammatory cytokines TNF-α, IL-1, IL-2, and IL-6 in the brain under diabetic conditions indicates that the innate immune system and microglial cells in particular are activated[77,78], and play an important role in neuronal damage in diabetic animals and patients[79,80]. Hyperglycemia, a defective insulin signalling system, and oxidative stress have been linked to neuronal toxicity and apoptosis, neuroinflammation, and the consequential development of neurodegeneration in diabetes[81,82].

T2DM AND NEURODEGENERATIVE AND NEUROVASCULAR DISEASES

There is growing evidence of a strong association between T2DM and neurodegenerative disorders such as Alzheimer's disease (AD) and neurovascular disorders[83,84]. Metabolic alterations, including central insulin resistance and abnormal glucose metabolism, are obvious in the mild cognitive impairment prodromal phase and in individuals that are still asymptomatic, but at increased genetic risk for AD [85]. Limited autopsy analyses suggest that hyperglycemia may promote AD pathology by inducing more prominent Aβ plaques and tau-positive cells accumulation, and activation of microglia in the comorbidity of AD and T2DM than in those patients with AD and without T2DM[86].

Recently, de la Monte and Wands[87] proposed a new term, type-3 diabetes or 'Brain-specific type-2 diabetes', for the neuroendocrine disorder that represents the progression of T2DM to AD[87,88] (Table 1). This state is characterized by decreased insulin production and insulin resistance[89]. The authors found that impairments of insulin-like growth factor signalling lead to these deficits in energy metabolism with increased oxidative stress, neuroinflammation, vascular damage, tau phosphorylation, Aβ accumulation, and neuronal degeneration[87,90]. In T2DM, islet amyloid polypeptide, also known as amylin, is secreted by pancreatic β-cells that modulate insulin and glucagon secretion and contribute to glucose regulation[91]. Islet amyloid polypeptide mainly affects cognitive function and causes blood-brain barrier (BBB) interruption, interacting and aggregating with Aβ peptides and hyperphosphorylates of tau protein within the brains of AD patients. Consequently, this leads to disruption in the neuronal network and neurodegeneration which could also be a link between T2DM and AD[92]. Inflammatory processes play a crucial pathogenic role in T2DM and AD[93]. A crosstalk between peripheral and central inflammation has been described[94]. Patel and Santani[95] showed that nuclear factor kappa B (NF-κβ) is involved in the inflammation of the brain during the progression of diabetes. NF-κβ also upregulates the expression of cytokines that are responsible for the insulin resistance onset, such are TNF-α, IL-1β, and IL-6[96,97]. These inflammatory mediators can cross the disrupted BBB and

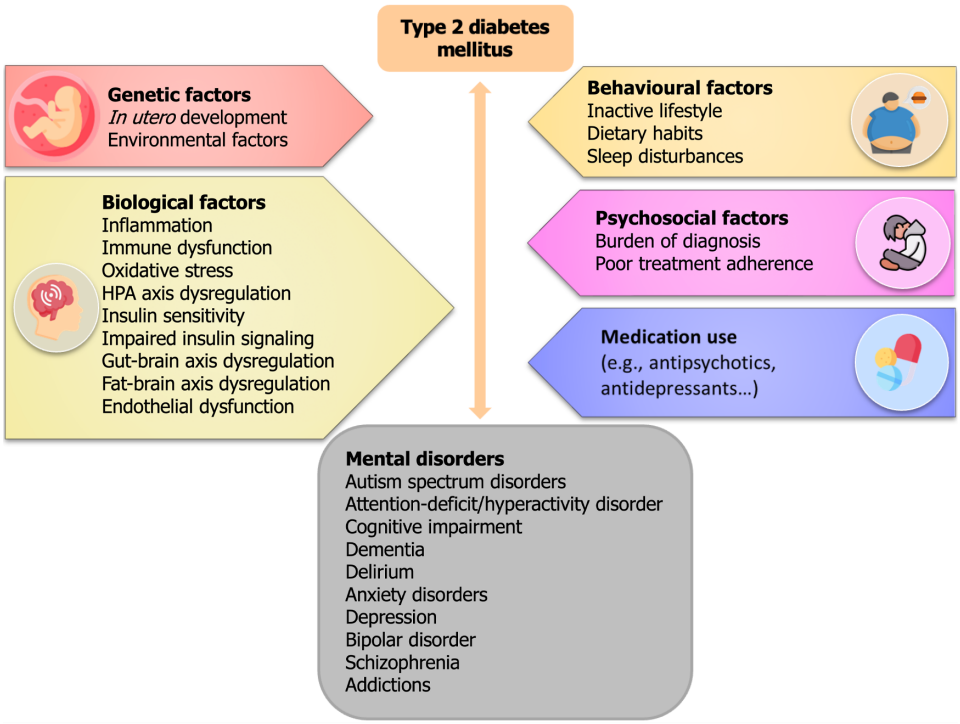

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Figure 1 The bidirectional link between mental disorders and diabetes mellitus type 2. In the co-occurrence of type 2 diabetes mellitus and mental disorders possible biological, psychological, and social factors should be considered. Various factors in intrauterine development and later life could exert their impact. Consequences are inflammatory and immune disturbances, oxidative stress, the hypothalamic-pituitary-adrenal axis dysregulation, gut-brain and brain-fat axis dysregulation, a complete presentation of metabolic syndrome, consequent endothelial dysfunction, *etc*. Individual behavioural and lifestyle patterns and applied treatment are of great importance in the onset of both entities. HPA: Hypothalamic-pituitary-adrenal.

enter the brain, further promoting neuroinflammation and leading to abnormalities of synapses, insulin resistance and damage of neural tissue, and eventually neurodegeneration[98-100]. Previous studies have reported that these proinflammatory cytokines are elevated in AD and found in amyloid plaques and their related glial cells[101].

T2DM is an established risk factor for neurovascular diseases such as ischemic stroke and cortical and subcortical microinfarcts[102]. Many studies report that cerebral infarcts are significantly associated with increased development of post-stroke cognitive impairment or vascular dementia[103,104]. The alterations in the glucose levels cause dysfunction and damage to the vessel's endothelium leading to atherosclerosis[105]. T2DM vascular complications affect the circulatory system in the brain by remodelling and stiffening the vascular walls, causing the reduction of vessel calibre with hypoperfusion[106]. Possible pathways of endothelial damage include oxidative stress and inflammation [107]. Chronic hyperglycemia and the production of reactive oxygen species apparently damage the vessel endothelium and lead to atherosclerosis[108]. In addition, damaged endothelial cells can release danger-associated molecular patterns (DAMP), activate toll-like receptor 4, and further potentiate inflammation[109]. The specific DAMP signals, the advanced glycation end products (AGEs), are proteins or lipids that become glycated as a result of exposure to elevated glucose concentration[110]. These molecules stimulate the receptor for AGEs (RAGE), CD36, and toll-like receptor 4 receptors which in turn stimulate inflammation, vascular injury, and oxidative stress[111]. RAGE is strongly expressed in microglia, astrocytes, and brain endothelial cells in T2DM[112,113]. Inflammatory signals can trigger local thrombotic vascular events leading to brain infarction[114] (all potential mechanisms summarized in Figure 1). The differential and relative contributions of T2DM, cerebrovascular and neurodegenerative disease to cognitive impairment and dementia are still unknown. Understanding the mechanisms and determinants of cognitive decline is of inestimable importance in future treatment strategies.

T2DM AND MENTAL DISORDERS

The study integrating data from transcriptomic meta-analysis of peripheral blood mononuclear cells and systems biology provided new insights into the shared pathogenetic mechanisms of schizophrenia and T2DM. This study showed that 28 genes concordantly dysregulated were included in the "positive regulation of catabolic process" pathway and low-grade inflammation, "membrane trafficking" particularly focused on clathrin-mediated endocytosis and "signalling by interleukins", transforming growth

factor beta and NF-κβ[115]. Schizophrenia as a neurodevelopmental condition is associated with a higher risk of T2DM also by common exposure to early life stress and alteration of fetal mental programming and immune-inflammatory dysregulation[116]. The association between drug-naïve first-episode schizophrenia and pre-diabetes conditions indicates an inherent risk for glucose regulation before antipsychotic treatment[117,118]. Parental history of diabetes was associated with the onset of diabetes in patients with schizophrenia that are treated with clozapine[119]. Treatment with second-generation antipsychotics has a 1.3-fold elevated risk of diabetes compared to first-generation antipsychotics[120].

Depression has also been shown to be nearly three and two times more common in patients with T1DM and T2DM, respectively[121]. When behavioural factors such as dietary habits, physical activity, socioeconomic status, and sleep are altered, they could lead to depression and T2DM. The relationship between a poor intrauterine environment and the risk of depression in adulthood is not clear, and there is no genetic association between T2DM and depression[122]. Habib *et al*[123] described shared etiological factors for the comorbidity between diabetes and depression, considering hypothalamic-pituitary-adrenal axis dysregulation and cortisol release, hyperactivity of the autonomic nervous system and catecholamines release, inflammatory processes, activation of the polyol pathway, inducing oxidative stress and increasing the formation of AGEs, and also damage *via* microvascular dysfunction. The bidirectional relationship between depression and diabetes is reflected in the psychological and psychosocial impact of depression, microvascular brain lesions, higher levels of glutamate, poor glycemic control, and medication compliance that could lead to diabetes, and conversely, the stress associated with diabetes management could lead to depression[124] (Figure 1). These mutual interactions are of particular clinical interest in vascular depression, a type of late-life depression that correlates with white matter hypersensitivity, which is also observed in patients with diabetes and associated depression[125].

Increased gut permeability links depression to T2DM when metabolic endotoxemia with lipopolysaccharides induces β-cell damage, and neuroinflammation[126,127]. Immune-inflammatory pathways, sterile inflammation, the release of DAMP, oxidative and nitrosative stress, and glia activation are also shared mechanisms. Non-alcoholic fatty liver disease is more common in people with mental disorders, including schizophrenia, major depressive disorder, and bipolar disorder, and is driven by the same lifestyle factors that put them at risk for T2DM[128].

The co-occurrence of diabetes and depression has more severe negative consequences. Individuals with depression and T2DM have a higher risk of cognitive decline and dementia compared with individuals treated for T2DM alone, which is important in clinical practice[129]. If clear causality is established, mental changes could certainly be prevented and cured. In a large cohort of Taiwanese diabetic patients, 0.8% of deaths were found to be due to suicide (0.14% of all patients)[130], and AbdElmageed and Mohammed Hussein[124] discussed different aspects of how suicide risk increased with elevated blood glucose levels and could be facilitated by patient access to potentially lethal agents such as oral hypoglycemics and insulin.

Martins *et al*[131] have concluded, based on an extensive literature review, that antidepressants may exert some positive effects on glycemic control in patients with DM. However, it is important to consider a specific subclass of anti-depressants or even different antidepressants of the same class, treatment duration, and the use of combination therapy. That being so, metabolic consequences need to be evaluated individually. Tricyclic antidepressants can worsen glycemic control, monoamine inhibitors may induce weight gain, and selective serotonin reuptake inhibitors are associated with the improvement in glycemic control. The antidepressant bupropion seems to improve glycemic control[132].

Enhanced release of dopamine by insulin is involved in the modulation of motivation and reward leading to depression symptoms[133]. Endocannabinoid system dysfunction could contribute to the development of depression in T2DM and could also be a therapeutical target[126]. On the other hand, antidiabetic drugs have a positive effect on the treatment of the major depressive disorder, by crossing the BBB and by mediating insulin signalling, inflammatory pathways, and cognitive performance. A group of distinguished authors has recently discussed that metformin may have beneficial effects not only in medical conditions but also in core illness domains in a wide range of psychiatric and neurodegenerative disorders[134]. Metformin, as an antihyperglycemic, appears to promote antidepressant, anxiolytic, and cognitive functions by increasing GLP-1, but also exerts anti-inflammatory effects by lowering C-reactive protein, inhibiting Th17 cell differentiation, and reducing TNF-β, IL-1β, IL-6, and IL-17. It also reduces oxidative and nitrosative stress, leading to an improvement in serotonergic neurotransmission in the hippocampus. The attractive new potential of metformin is to protect the intestinal barrier and modulate BBB function. It is worth noting that leptin crosses the BBB and binds to receptors that are spread in different brain areas and seem to have antidepressant and anxiolytic properties[135].

CONCLUSION

The relationship between T2DM and psychiatric disorders demonstrates how our mental and physical

health are inevitably intertwined. The mechanisms underlying this bidirectional relationship remain unresolved, with various intriguing hypotheses. Common biological mechanisms that may underlie both diabetes and psychiatric disorders represent the basic goals of future research. Shared genetic pathways could be a potential explanation, but data from existing studies are still insufficient to draw definitive conclusions. Of particular interest are the possible overlaps in genetic mechanisms between schizophrenia and T2DM. Intrauterine development represents the initial and unavoidable starting point for the predisposition to numerous pathological conditions after birth. Inflammation is another likely suspect underlying both diabetes and psychiatric disorders. A better understanding of the gut-brain axis and its complex relationship with the gut microbiome is essential for developing new therapeutic strategies to combat both diabetes and psychiatric disorders.

Given the burden of diabetes and concomitant cognitive changes and psychiatric diseases, it is a crucial need to understand the complex multifactorial pathophysiology of DM and to identify molecular targets and pathways that might lead to future therapies. The potential of integrated approaches needs to be thoroughly explored in future trials. In the clinical arena, the early evaluation and accurate quantification of cognitive functions and mental state need to be implemented in the clinical assessment of diabetic patients at the very beginning as well as on follow-up on a regular basis, as it significantly impacts the complete recovery and quality of life these patients. Vice versa approach should also be applied. Translational application of anti-glycemic drugs in the treatment of depression and dementia could be a useful path in the future. All this could jointly direct future interventions to improve the outcome of somatic treatment and better quality of life in persons with mental disorders.

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REVIEW

Mechanism of immune attack in the progression of obesity-related type 2 diabetes

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Abstract

Obesity and overweight are widespread issues in adults, children, and adolescents globally, and have caused a noticeable rise in obesity-related complications such as type 2 diabetes mellitus (T2DM). Chronic low-grade inflammation is an important promotor of the pathogenesis of obesity-related T2DM. This proinflammatory activation occurs in multiple organs and tissues. Immune cell-mediated systemic attack is considered to contribute strongly to impaired insulin secretion, insulin resistance, and other metabolic disorders. This review focused on highlighting recent advances and underlying mechanisms of immune cell infiltration and inflammatory responses in the gut, islet, and insulin-targeting organs (adipose tissue, liver, skeletal muscle) in obesity-related T2DM. There is current evidence that both the innate and adaptive immune systems contribute to the development of obesity and T2DM.

Key Words: Type 2 diabetes mellitus; Obesity; Insulin resistance; Immune cells; Inflammation; Pathological mechanism

Core Tip: Obesity is closely associated with the occurrence and development of insulin resistance and type 2 diabetes mellitus (T2DM). Previous studies have demonstrated the important role of immune cell infiltration and inflammatory response in obesity-related T2DM. This review presents immune responses in the gut with respect to metabolic challenges. We also highlight the effects of immune attacks and proinflammatory shifts on insulin-secreting and targeting organs.

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INTRODUCTION

Globally, obesity and associated complications are widespread. Over the past 40 years, the impact of this non-contagious disease has spread from high-income countries to low- and middle-income countries, with its prevalence nearly tripling globally. Statistics from the World Health Organization in 2016 showed that 13% of the global adult population is obese, and more than 1.9 billion adults are overweight. The prevalence and degree of overweight and obese children and adolescents have also noticeably risen, generating concern for future years. Up to 2025, it is estimated that about 20% of the global population will be obese[1,2]. Widespread obesity among adults and adolescents will lead to a striking increase in obesity-driven health complications such as type 2 diabetes mellitus (T2DM), as most T2DM patients tend to be overweight or obese[3,4].

The close correlation of obesity with T2DM has generated broad research interests of researchers. Although the pathophysiological mechanisms linking obesity to T2DM remain unclear, many studies have suggested that immune attack induced by overnutrition in multiple organs strongly contributes to insulin resistance (IR), lipotoxicity, and glucotoxicity. In this review, we examine recent advances and underlying mechanisms of local and systemic immune attack and chronic low-grade inflammation in T2DM induced by obesity.

IMMUNE ATTACK IN THE GUT OF OBESITY-RELATED T2DM

Most patients with T2DM are obese or overweight. These two states represent the disrupted condition of energy homeostasis in the body, due to chronic excessive calorie intake over expenditure. The gut is the first important "station" through which high-calorie food enters the body. There is recent widespread evidence that disturbance to the gut (particularly the dysbiosis of gut microbiota, imbalance of immune cells, and impaired gut barrier function) hinders the immune response and contributes to the development of obesity related IR and T2DM (Figure 1).

The composition of gut microbiota is complex, with high variability across individuals. This composition can be altered by changes to diet, and is closely associated with the development of disease. Reduced gene richness of gut microbiota is a common phenomenon caused by modern dietary structure, and might be associated with dyslipidemia, severe IR, and low-grade local or systemic inflammation[5,6]. Existing studies have shown that after introducing microbiota from obese donors to germ-free mice, lipid accumulation and IR arose. This result demonstrated the close association between the gut microbiota and metabolic disorders in obesity-related T2DM[7,8]. Changes to metabolites caused by an altered gut microbiome help mediate the link between the host and gut microbiome. Short-chain fatty acids (SCFAs) are the products of undigested dietary fibers degraded by gut bacteria, and include acetate, propionate, and butyrate. These SCFAs have anti-inflammatory properties, in particular, butyrate[9,10]. Metagenome-wide studies have shown that the dysbiosis of gut bacteria occurs in patients with T2DM, in which the abundance of butyrate-producing bacteria declines, while that of opportunistic pathogens increases[11,12]. For instance, the administration of commercial *Bifidobacterium* strains reduces body weight gain and downregulates inflammation, by reshaping intestinal gene signatures in mice[13]. Many studies have shown that the anti-inflammatory effects of butyrate are mainly achieved by inhibiting mitogen-activated protein kinase pathways and nuclear factor kappa B (NF-κB) in intestinal epithelial cells, which reduce the secretion of proinflammatory mediators and molecules involved in the homing of inflammatory cells[14]. The metabolite-sensitive G protein-coupled receptor (GPR) and its ligands strongly affect anti-inflammatory responses, with SCFA functioning being partially mediated by their receptors GPR41 and GPR43[15-17]. In addition to SCFAs, bacteria from the phylum *Bacteroidetes* produce glycan from fiber modulating immune function to protect against inflammation, such as polysaccharide A and peptidoglycan[18]. Thus, the anti-inflammatory responses involving SCFAs and other microbial-related metabolites in the intestine are likely weakened

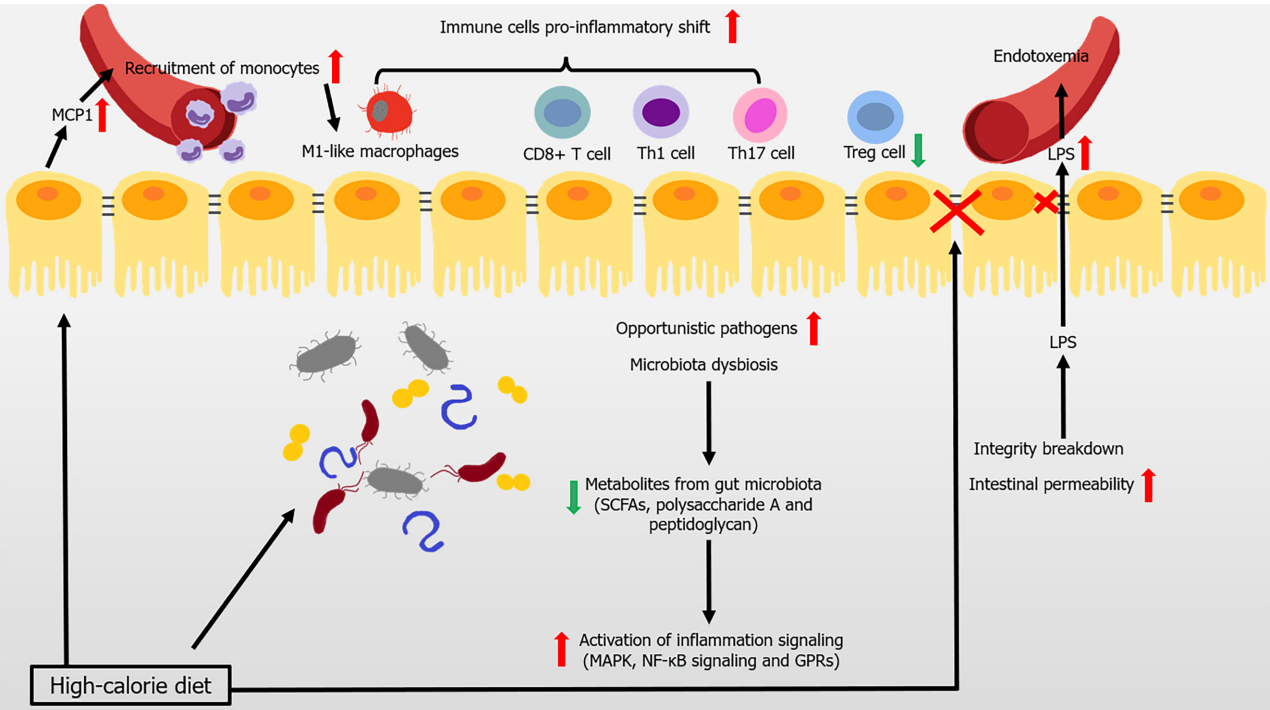

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Figure 1 Immune attack and inflammation in the gut during obesity-related type 2 diabetes. In the context of obesity and type 2 diabetes mellitus, overnutrition leads to the reduced gut microbiota, and even the increase of opportunistic pathogens. At the same time, the occurrence of decreased metabolites levels with anti-inflammatory effects, is accompanied by the activation of inflammation signaling. During obesity, imbalance of pro- and anti-inflammatory immune cells occurs in the gut. The intestinal epithelial cell-produced monocyte chemoattractant protein-1 (MCP1) recruits the circulating monocytes to the gut and they shift to the pro-inflammatory phenotype. High fat diet also induces a pro-inflammatory shift in T cells, accompanied with decreased regulatory T cells. Immunoglobulin A (IgA)-secreting immune cells and IgA secretion are both decreased. High-calorie diet and several recruited immune cells also impair intestinal barrier and increase intestinal epithelial and gut vascular permeability, leading to the leakage of microbiota-derived molecules (such as lipopolysaccharide [LPS]) into blood. High levels of LPS and other bacterial products cause endotoxemia and inflammation in multiple organs that further aggravate the metabolic diseases. GPR: G protein-coupled receptor; SCFAs: Short-chain fatty acids.

in the gut, and are likely closely associated with the development of obesity and T2DM.

The infiltration and proinflammatory shift of immune cells contribute to the inflammation of the intestine under metabolic challenge. In mice and obese humans, high-fat diet (HFD) induces chemokine (C-C motif) ligand 2/monocyte chemoattractant protein-1 (CCL2/MCP-1) production to rise in epithelial cells, which recruit monocytes to the gut, shifting to the proinflammatory phenotype[19,20]. Macrophage-specific deletion of C-C chemokine receptor type 2 (CCR2) ameliorates insulin sensitivity and glucose tolerance, confirming the association between the infiltration of proinflammatory macrophages and obesity-induced metabolic disorders[19]. Moreover, HFD also induces a proinflammatory shift in T cells, with elevated interferon gamma (IFN-γ)-producing CD4+, CD8+ T cells, and interleukin 17 (IL-17)-producing γδ T cells, along with decreased regulatory T cells (Tregs)[21]. Tregs are one lineage of CD4+ T cells. These cells are involved in maintaining immune homeostasis and restricting excessive immune responses. T helper 17 (Th17) cells might secrete IL-17A, IL-17F, IL-21, and IL-22. Several strains of *Clostridia* help with the expansion and differentiation of Tregs, by providing bacterial antigens and an environment rich in transforming growth factor beta, contributing to the immunological homeostasis of the gut[22,23]. *Lactobacillus reuteri, Bacteroides fragilis*, *B. hetaiotaomicron*, *Clostridium*, and *Faecalibacterium prausnitzii* promote the differentiation of Tregs. Segmented filamentous bacteria are required for Th17 cells to develop in the gut. Furthermore, SCFAs improve the Treg/Th17 balance, and induce IL-22 production in CD4+ T cells and innate lymphoid cells (ILCs), maintaining intestinal homeostasis[17,24,25].

Many studies have shown that serum lipopolysaccharide (LPS) levels rise in T2DM patients, with a triggering factor to IR and diabetes being identified that is closely associated with intestinal integrity and permeability[26,27]. One recent study of 128 obese human subjects showed that the abundance of *Escherichia coli*, an important producer of LPS, was higher in obese patients with T2DM compared with the lean patients[28]. LPS is recognized by Toll-like receptors (TLRs) of the innate immune system, leading to the aggregation of macrophages and activation of the NF-κB inflammatory signaling pathway. This process triggers systemic immune and inflammatory responses that aggravate IR[14,29]. In general, a healthy intestinal barrier protects the organism from the passage of microbes. However, the intestinal barrier of people with T2DM is disturbed, leading to the uncontrolled passage of LPS and microbiota-derived molecules, and subsequent endotoxemia and chronic inflammation[30]. In

particular, obese mice have fewer immunoglobulin A (IgA)-secreting immune cells and lower IgA secretion and glucose metabolism disorders arise in obese IgA-deficient mice. Administering metformin and bariatric surgery augment cellular and stool IgA levels[31]. Obese patients with T2DM exhibit a lower expression of intestinal tight junction genes and interference with the WNT/β-catenin signaling pathway, both of which are linked increased intestinal epithelial and gut vascular permeability[31-33]. Several immune cells (such as mucosal-associated invariant T cells [MAIT]) also impair gut integrity by inducing the dysbiosis of microbiota[34]. IL-1β can increase barrier permeability in intestinal epithelial cells, whereas IL-22 is considered a protector of maintaining intestinal barrier integrity[35-37]. Reduced integrity and higher intestinal permeability of the intestine promote the translocation of microbiota-derived molecules from the intestinal lumen to the bloodstream. This process triggers the activation of lamina propria macrophages in the intestine, causing LPS levels to rise in the blood.

IMMUNE ATTACK IN THE ADIPOSE TISSUES OF OBESITY-RELATED T2DM

Eating more calorie-dense foods combined with less exercise promotes the development of obesity. In both mice and humans, excess energy is stored in white adipose tissues (ATs) (WAT), which serves as the immune and endocrine organ containing mature adipocytes, adipocyte precursor cells (also called adipose stromal cells), and immune cells. Obesity causes a persistent low-grade inflamed condition in these expanding adipose depots, and the simultaneous infiltration of immune cells in the stromal vascular fraction and systematic metabolic disorders. The inflammatory storm driven by dysfunctional WAT disrupts its normal function and that of other insulin-sensitive organs. Consequently, this process contributes to the pathophysiological mechanisms of IR and T2DM (Figure 2). However, in obese subjects with T2DM, this immune attack appears to be stronger. Obese patients with T2DM have a higher degree of inflammation at both the systemic and AT level compared to patients with normal glucose tolerance. This phenomenon is characterized by aggravated macrophage infiltration in WAT, with elevated IL-6 levels and CD4+ T cell numbers in serum[38].

Macrophages are representative immune cells of the innate immune system, and were first studied in relation to the process of immune infiltration in WAT. The infiltration and activation of macrophages is beginning to be recognized as a pivotal instigator of meta-inflammation. Normally, M2 anti-inflammatory macrophages are the main type in WAT[39,40]. However, as obesity develops, instead of the M2-phenotype, M1 proinflammatory macrophages in AT gradually increase (up to 40% of cells in AT), leading to a proinflammatory state in WAT[40-42]. The greater increase in M1-like polarized macrophages results in their being responsible for almost all secretions of tumor necrosis factor alpha (TNF-α) and IL-6 in WAT. In turn, this process impairs the insulin signaling pathway, leading to IR, both locally and systemically[43]. Initially, the proliferation of resident macrophages dominates the accumulation of macrophages in WAT. Then at the later stage of obesity, recruited monocytes con-tribute to the accumulation of macrophages, following the secretion of CCL2/MCP-1 and leukotriene B4 by adipocytes to the microenvironment[44-46]. Free fatty acids (FFAs) derived from the diet and triglyceride lipolysis in hypertrophied adipocytes also promote M1-like polarization through a TLR4-dependent mechanism in WAT. In turn, this process increases FFA levels by aggravating lipolysis, establishing a positive feedback loop between FFAs and TLR4 activation in WAT[47,48]. Moreover, microRNAs (miRNAs) are considered to be important links between adipocytes and macrophages. Adipocyte-derived miRNAs (such as miR-30, miR-34a, miR-21, and miR-10a-5p) regulate the immune balance between M1- and M2-macrophage polarization[49-52]. Besides, proinflammatory macrophages also facilitate neutrophil recruitment to metabolic tissues during obesity, by releasing nucleotides through pannexin-1[53].

Aside from macrophages, adaptive immune cells are involved in the pathogenesis of obesity-related T2DM. In HFD-induced obese mice, CD8+ T cells are recruited into AT, promoting M1-like polarization [40,54,55]. However, different categories of CD4+ T cells have various functions in AT[56]. Proinflammatory CD4+ T cells (Th1, Th17, and Th22) are important promoters of the development of obesity-associated metabolic disorders. These cells produce proinflammatory cytokines (IFN-γ, TNF-α, IL-17, and IL-22), and are involved in the recruitment and activation of M1 macrophages[57-60]. MAIT are innate-like T cells that express a semi-invariant T cell receptor, which promote inflammation in AT by inducing M1 macrophage polarization. This process leads to IR and impaired glucose and lipid metabolism[34]. Conversely, Tregs provide an essential accessory function that prevents systemic metabolic disorders, through suppressing the expression of MCP-1 in adipocytes to limit M1 macrophage infiltration *via* IL-10 and other insulin-sensitizing factors. However, the development of Tregs in WAT seems to depend on insulin signaling. Insulin signaling drives the transition of $CD73^{lo}ST2$ (IL-33 receptor) ^{hi}adipose Treg subsets, which might also suppress inflammation in WAT *via* the hypoxia inducible factor 1 alpha–mediator complex subunit 23–peroxisome proliferator-activated receptor gamma axis[61]. Furthermore, AT B cells also negatively control local inflammation by secreting IL-10 (secreted by Bregs) and other soluble factors. B cells also contribute to systemic inflammation by activating CD8+ and Th1 cells, and releasing pathogenic antibodies[62-65]. B cells from obese mice consistently produce a proinflammatory cytokine profile compared to those from lean controls[66]. B

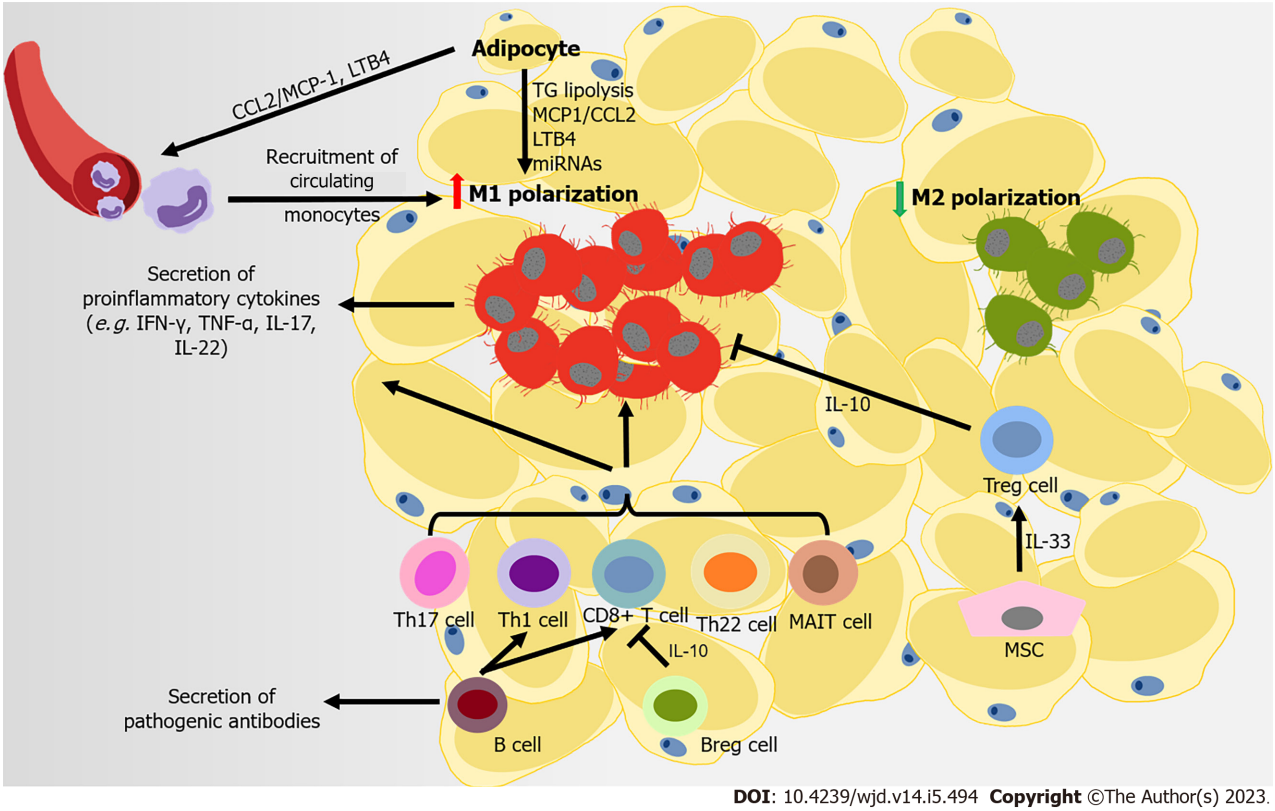

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Figure 2 Immune attack and inflammation in the white adipose tissue during obesity-related type 2 diabetes. At the later stage of obesity, recruited monocytes mainly contribute to the accumulation of macrophages in adipose tissue, following the secretion of monocyte chemoattractant protein-1 (MCP1) and leukotriene B4 (LTB4) by adipocytes to the microenvironment. Free fatty acids from the diet and in triglyceride (TG) lipolysis in adipocytes promote M1-like polarization. Several adipocyte-derived microRNAs also regulate the immune balance between M1- and M2-macrophage polarization. CD8+ T cells, pro-inflammatory CD4+ T cells (T helper type 1 [Th1], Th17, and Th22) and mucosal-associated invariant T cells are also recruited into adipose tissue, promoting M1-like polarization. Regulatory B cells (Bregs) and regulatory T cells (Tregs) can negatively control the local inflammation by secreting interleukin-10 (IL-10), but B cells contribute to systemic inflammation by activating CD8+ and Th1 cells, and releasing pathogenic antibodies. Some mesenchymal stromal cells in visceral adipose tissue can improves insulin resistance and inflammation in adipose tissues through expanding and sustaining the resident Treg population *via* IL-33 secretion.

cells transferred from obese mice induce the development of IR in B cell-deficient lean mice. By contrast, B cell depletion in mice restores aberrant immune cell composition and improves metabolic capacity in WAT[67]. T-bet B cells are B cells lacking cluster of differentiation 21 (CD21) and CD23. These cells accumulate in humans that have an elevated body mass index, and in mice with higher body weight. Mice without T-bet B cells have lower weight gain and M1 macrophage infiltration in WAT[68,69]. Thus, regulation of the adaptive immune system is related to the inflammation of AT in obesity. Adaptive immune cells are involved in AT IR in obesity-related T2DM; however, some of these effects may be achieved through promoting the polarization of M1-like macrophages.

Recent studies have shown that other types of cells in AT also participate in regulating immune balance. Mesenchymal cells contribute towards shaping immune responses and maintaining immune homeostasis in WAT. Mesenchymal cells express IL-7, IL-33, and CCL19, which recruit both innate and adaptive lymphocytes. IL-33 is produced by particular mesenchymal stromal cells in visceral AT (VAT), IL-33 improves IR and inflammation in AT, possibly through expanding and sustaining the resident Treg population[70-73]. Administering IL-33 helps combat obesity, by markedly increasing the fraction of group 2 ILCs and eosinophil, and improving WAT browning[74].

However, the distribution of AT appears to be closely related to the occurrence and progression of metabolic diseases. It has been universally accepted that central body fat deposition and injured function of AT are closer associated with obesity-related metabolic diseases than fat mass in the whole body. Generally, AT is divided into abdominal subcutaneous AT, femoral subcutaneous AT (FSAT, main type of lower-body AT), VAT, according to their different location. SAT is the largest AT depot. The expansion of FSAT and adipocyte hyperplasia from precursor cells are considered to be a healthier alterative of AT in meeting elevated storage energy demands. However, any damage to these approaches leads to the accumulation of fat in upper body AT and organs, which causes “lipotoxicity” in other insulin-sensitive organs, as well as systemic IR and a higher risk of T2DM. Several studies have found that SAT may have a more beneficial metabolic phenotypes, notably its accumulation in lower-body[75,76]. Upper body AT (especially VAT) is usually characterized by more rapid storage of energy and a higher lipolysis rate than lower-body, which contributes to systemic FFA levels[77]. Interestingly,

a recent study revealed that expanded adipocytes, lower SAT oxygenation, inflammation infiltration in SAT, and elevated FFA release, these changes in SAT that were considered harmful, seemed to be unrelated to the occurrence of obesity-induced IR[76,78]. Collectively, expansion and inflammation in VAT, rather than SAT, are the culprit involved in obesity-related metabolic diseases. Therefore, the effects of abdominal WAT accumulation are of more concern.

INFLAMMATION AND IMMUNE STATUS IN METABOLICALLY HEALTHY OBESITY

Metabolically healthy obesity (MHO) is a subgroup of obesity, which does not have an universally accepted definition. In most studies, MHO presented without the following features: dyslipidemia, IR, impaired glucose metabolism, and overt T2DM. Compared with metabolically unhealthy obesity (MUO), MHO usually has more expandability of SAT, less ectopic fat accumulation, normal concentration of inflammatory markers, and preserved better β-cell function, and insulin sensitivity[79-81]. Systematically, decreased concentrations of C-reactive protein, TNF-α, IL-6, and plasminogen activator inhibitor-1 were found in the MHO subjects than MUO individuals[82]. Changes to the distribution and function of AT might also strongly contribute to the conversion of these two states. Excess caloric storage demand leads to the overload of SAT and ectopic fat accumulation and this ectopic fat deposition will eventually cause the transition from MHO to MUO[79]. Besides, many studies have revealed that less immune cells infiltration (such as proinflammatory macrophages and T lymphocytes) and cytokines production in MHO than in MUO, usually along with the increased VAT mass[83-86]. Improved antioxidant capacity and diminished oxidative stress could be also observed in MHO subjects than in MUO people[87,88].

IMMUNE ATTACK IN THE LIVER OF OBESITY-RELATED T2DM

The liver is the metabolic center of nutrients and drugs in the body. It receives material supplied from the gut *via* the portal vein, proinflammatory immune cells and cytokines from circulation, which strongly impact its physiological function (Figure 3).

Liver macrophages contribute to obesity-related hepatic IR by producing both inflammatory and non-inflammatory factors. Hepatic macrophages include resident macrophages (Kupffer cells [KCs], high expression of F4/80 and C-type lectin domain family 4 member F) and recruited hepatic macrophages (RHMs), high expression of CD11b and CCR2. RHMs are derived from circulating Lyc6+ monocytes, which are recruited by steatosis hepatocytes and KCs secreting CCL2/MCP-1[89-92]. Although the ratio of KC to RHM is different in the liver of healthy mice and humans, as obesity develops, hepatic RHMs noticeably increase. These RHMs serve as a main promoter of inflammation injury in the liver, by producing chemokines and cytokines (in both humans and mice), which are related to obesity induced IR[93-95]. Multiple mechanisms are involved in the proinflammatory activation of hepatic macrophages. In obese individuals, FFAs overflow from obese AT contributes to the activation of resident hepatic macrophages[96]. Leptin and adiponectin from expanded AT have contrasting actions on KCs. The former stimulates proinflammatory and profibrogenic cytokines in KCs, whereas the latter modifies KCs towards anti-inflammatory phenotypes[97,98]. AT-derived proinflammatory cytokines (such as IL-1β) contribute to the chronic activation of hepatic NF-κB, promoting the development of nonalcoholic steatohepatitis (NASH)[99]. KCs highly express scavenger, complement, and pattern recognition receptors, including TLRs. Intestinal permeability rises during obesity, leading to the translocation of bacteria or their products to the portal circulation. These substances are recognized by TLRs in macrophages, which activate NF-κB, IFN regulatory factors and other downstream transcriptional factors to induce inflammatory responses[100]. Microbe-related products, including extracellular vesicles (mEVs) containing gut microbial DNA, that leak from gut reach the liver, and exacerbate obesity-associated hepatic inflammation and IR. Vsig4+ macrophages and CRIg+ macrophages efficiently clear mEVs through a complementary component C3-dependent mechanism; however, HFD impairs these benefits[101,102]. CD68 serves as a marker for macrophages residing in the liver; however, this indicator is not sufficient for distinguishing them from monocyte-derived cells. The utilization of single-cell sequencing allows their origin, function, and associated inflammatory phenotype to be clearly distinguished. Two distinct populations of intrahepatic CD68 macrophages exist in human livers. *CD68MARCO*$^{+++-}$ cells are characterized by the enriched expression of LYZ, CSTA, and CD74, which represent their proinflammatory function. The *CD68MARCO* macrophage subset is similar to resident KCs, inducing immune tolerance[103]. Counter to expectation, KCs in diet-induced steatohepatitis probably participate in reparation pathways, not proinflammatory function[104]. However, KCs and RHMs both shift towards a proinflammatory phenotype[105]. Overall, the types and functions of liver macrophages are still under investigation.

Nonalcoholic fatty liver disease (NAFLD), obesity, and T2DM are closely related in terms of pathogenesis. The prevalence of NAFLD is higher in subjects with obesity compared to lean subjects [106,107]. T2DM is also closely associated with NAFLD and its severe form NASH. Most T2DM patients

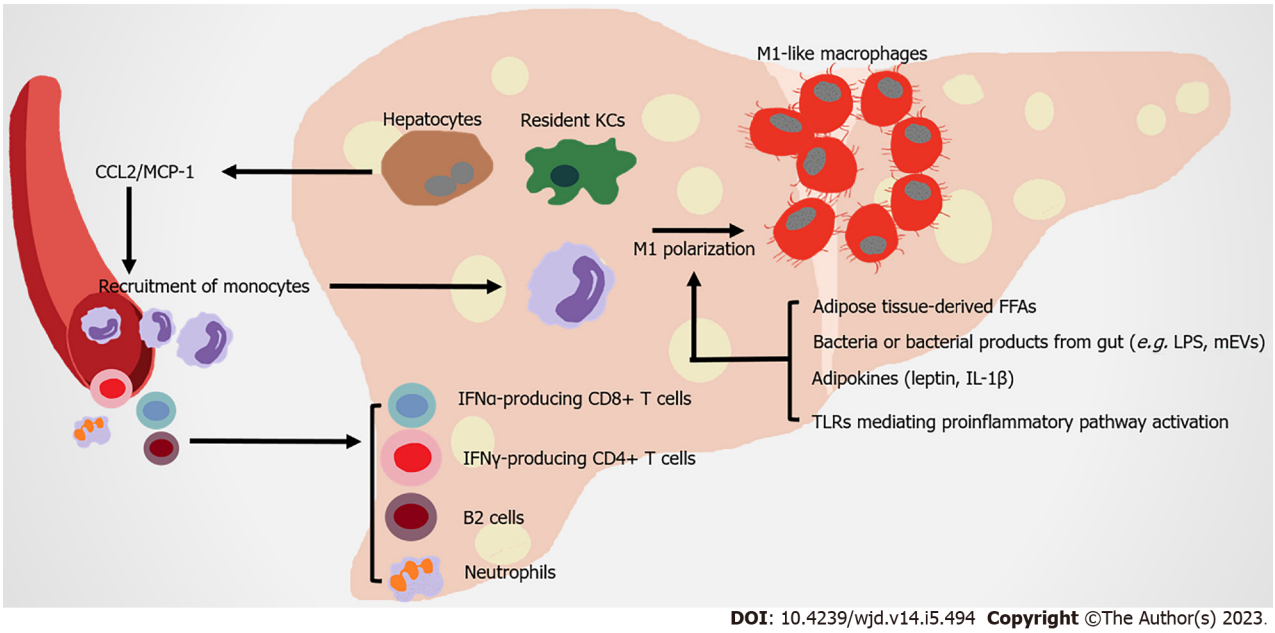

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Figure 3 Immune attack and inflammation in the liver in obesity-related type 2 diabetes. Under metabolic stress, recruited hepatic macrophages, which are derived from circulating monocytes, are recruited by steatosis hepatocytes and Kupffer cells secreting monocyte chemoattractant protein-1 (MCP1). Expanded adipose tissue-derived free fatty acids, leptin, interleukin-1 beta (IL-1β) and bacteria with their products from gut, contribute to the M1 polarization of hepatic macrophages. Nonalcoholic steatohepatitis (NASH) is a severe form of nonalcoholic fatty liver disease, which is associated with more severe hepatic insulin resistance and inflammation. The infiltration of neutrophils, B2 cells, interferon gamma (IFN-γ)-producing CD4+ T cells and IFN-α-producing CD8+ T cells occur in NASH liver, promoting insulin resistance under diet-induced metabolic stress. FFAs: Free fatty acids; KCs: Kupffer cells; LPS: Lipopolysaccharide; mEVs: Extracellular vesicles.

suffer from NAFLD[108-110]. NAFLD, particularly NASH, usually leads to more severe hepatic IR that negatively affects T2DM development[111]. In NASH mice, KC is gradually replaced by RHM. Although RHM could respond to local environmental clues and develops a KC-like transcriptomic profile, this profile is not identical to original healthy KCs[90]. In healthy subjects, KCs inhibit monocyte and macrophage recruitment by secreting IL-10 and promoting immune tolerance through inducing Tregs and programmed death-ligand 1 expression. However, when NASH happens, injured hepatocytes activate KCs and recruit monocytes to the liver, and produce proinflammatory cytokines. Besides, these proinflammatory macrophages trigger the activation of hepatic stellate cells, leading to progression of the extracellular matrix and fibrosis in liver[112,113]. TLRs mediate the greater activation of the proinflammatory pathway as NASH progresses. Excess FFAs drive the endocytosis of a monomeric TLR4 complex, enhancing the generation of reactive oxygen species and causing steatohepatitis and IR[114]. TLR2 and TLR4 signaling activates inflammasomes (*e.g.*, pyrin domain-containing protein 3, NLRP3) in KCs, aggravating hepatic steatosis and NASH inflammation[115-117]. TLR9 is primarily confined to the endosomes of macrophages, which are activated by higher levels of mitochondrial DNA and oxidized DNA in liver, triggering NASH[118,119]. Conversely, inhibition of TLR2, TLR4, and TLR9 signaling pathways has anti-inflammatory effects, representing a potential treatment target for NASH[118,120].

The histopathology hallmarks of human NASH include the infiltration of neutrophils with MPO-positive immunoreactivity[99]. Neutrophil extracellular traps (NETs) are extracellular web-like structures of decondensed chromatin with cytosolic and granule proteins. These structures are important in hepatic chronic inflammatory conditions. NET blockade significantly decreases the infiltration of RHMs and neutrophils[121].

Moreover, recent studies have focused on elucidating the role of adaptive immunity cells in liver inflammation under metabolic challenge. The accumulation of B cells (especially B2 cells) and T cells in liver arises in more than half of NASH patients[122-124]. B cell-activating factor levels in the circulation are elevated in NASH patients compared to those with simple steatosis. This phenomenon is associated with more advanced IR, more severe steatohepatitis and fibrosis[123,125,126]. The contribution of B cells to the progression of NASH could be attributed to the production of proinflammatory mediators and their antigen-presenting capabilities[122]. Interfering with B2 cells reduces the Th1 cell activation of liver CD4+ T cells and IFN-γ production[123]. In both humans and mice, IFN-γ-producing CD4+ T cells and IFN-α-producing CD8+ T cells increase in the liver, promoting IR under diet-induced metabolic stress[127,128]. Thus, the infiltration of adaptive immunity cells in liver strongly affect inflammatory mechanisms during the development of NASH.

IMMUNE ATTACK IN THE ISLET OF OBESITY-RELATED T2DM

In the pathophysiologic process of islets of obesity and T2DM, innate immune cells are important, especially macrophages. Increased infiltration of resident macrophages and transformation towards a proinflammatory phenotype contributes to obesity and T2DM islets, the extent of which is generally correlated with β-cell dysfunction[129-131] (Figure 4). Islet macrophages express F4/80, CD11c, major histocompatibility complex class II, CD64, CD11b, CX3C motif chemokine receptor 1, CD68, and lysozyme[132]. At the early stage of obesity, resident macrophages enhance the compensatory proliferation of β cells, mediated by platelet-derived growth factor (PDGF)-PDGF receptor signaling[129]. As the disease progresses, CD68-positive macrophages are elevated in T2DM islets[130,133,134]. The proliferation of resident macrophages causes them to accumulate in islets with elevated inflammatory cytokines and chemokines (such as IL-1β, TNF-α), impairing the hyperplasia and dysfunction of β cells [131]. Overall, changes to the number and function of islet macrophages affect the pathogenesis of obesity and T2DM.

However, the factors that trigger the infiltration and proinflammation polarization of macrophages in islets remain unclear. B cells are potentially one of the early responders in the altered islet microenvironment. In obesity, β cells recruit Ly6C+ monocytes to the islets by producing chemokines, despite these recruited monocytes remaining at the boundary of the exocrine and endocrine pancreas[129]. Amyloid deposition in islets is a typical pathological feature of T2DM, and is also a strong stimulus for macrophage-mediated NLRP3 inflammasome activation and IL-1β production[135-137]. In amyloid-positive T2DM islets, the number of macrophages greatly increases, with CD68 and inducible nitric oxide synthase-positive[134]. Macrophages that are resident to islets act as heightened sensors of interstitial ATP levels. Consequently, glucose-activated insulin and ATP co-secretion of β cells might trigger cytokine production from macrophages[138]. Macrophages resident to islets are in contact with blood vessels, probably protecting against inflammatory moieties from blood by extending their filopodias; however, high concentrations of glucose in T2DM limit this method of capture[139,140]. In addition, GRP92 activation in islet macrophages promotes conversion to the anti-inflammatory phenotype, and improves β-cell function[141]. The accumulation of intestinal mEVs causes CD11c+ macrophages to increase, with elevated IL-1β in islets impairing insulin secretion. Vsig4+ macrophages in islets block intestine-derived mEV *via* a C3-mediated mechanism. By contrast, obesity causes a marked decrease in Vsig4+ macrophages[142].

IL-1β is a key proinflammatory cytokine that clearly increases in T2DM islets. Although macrophages are considered to be the major producers of IL-1β in obesity islets, for which the potential mechanism has been identified, β cells also produce IL-1β[129,137]. Glucose-induced IL-1β auto-stimulation in β cells might contribute to glucotoxicity in T2DM islets[143,144]. However, IL-1β on β cells seem to have varied effects. For instance, low concentrations of IL-1β help to increase β-cell proliferation and improve insulin secretion following glucose stimulation. By contrast, high concentrations of IL-1β promote inflammation in islets, and might be closely related to the development of pre-diabetes and T2DM[145-147]. The IL-1R antagonist (IL-1Ra) also declines in T2DM β cells, pushing the IL-1/IL-1Ra balance towards a proinflammatory state[148-151]. The vaccine and responsive miRNA targeting IL1β are promising approaches for treating T2DM, by restoring β-cell mass, inhibiting β-cell apoptosis, and increasing insulin secretion[152-154]. Thus, antagonizing IL-1β is a potential target for T2DM treatment.

IMMUNE ATTACK IN THE SKELETAL MUSCLE OF OBESITY-RELATED T2DM

As skeletal muscle is the principle organ for glucose disposal, IR in this tissue becomes a crucial determinant of obesity and T2DM-related metabolic disorders[155,156]. Immune attack and inflammatory responses in skeletal muscle also regulate IR formation (Figure 5). CD11c-expressing proinflammatory macrophages, monocytes, and neutrophils are higher in the skeletal muscle of HFD-induced mice compared to the control[157,158]. More macrophages markers are found in the skeletal muscle of healthy subjects after HFD administration, with the development of IR[159,160]. In obese T2DM patients, the number of CD68+ macrophages is elevated in skeletal muscle[158,161]. Total T cells and αβ T cells, containing CD8+ T cells and IFN-γ–producing CD4+ cells are higher in the skeletal muscle of obese mice compared to control mice[162]. FFAs induce or synergize with macrophages to aggravate the inflammatory response in muscle cells, resulting in IR[163-165]. These immune cells infiltrate skeletal muscle, and accumulate in muscle AT between myocytes and the surrounding muscle, leading to higher levels of local proinflammatory cytokines, such as TNF-α, IL-1β, and IFN-γ[158,160,166].

Similar to adipocytes, skeletal muscle cells produce MCP-1, IL-6, IL-8, TNF-α, and other molecules, and part of these molecules lead to the infiltration of macrophages, inducing IR[157,167]. Muscle biopsies show that the gene expression of inflammatory cytokines (such as TNF-α) is upregulated in IR subjects[168]. Compared with non-diabetic subjects, more IL-6, IL-8, IL-15, TNF-α, growth related oncogene α, MCP-1, and follistatin are released by skeletal muscle cells from T2DM patients[169]. Aerobic exercise reduces the infiltration of macrophage in skeletal muscles, and improves insulin sensitivity and elevates the production of anti-inflammatory cytokine IL-10[170]. IL-10 attenuates

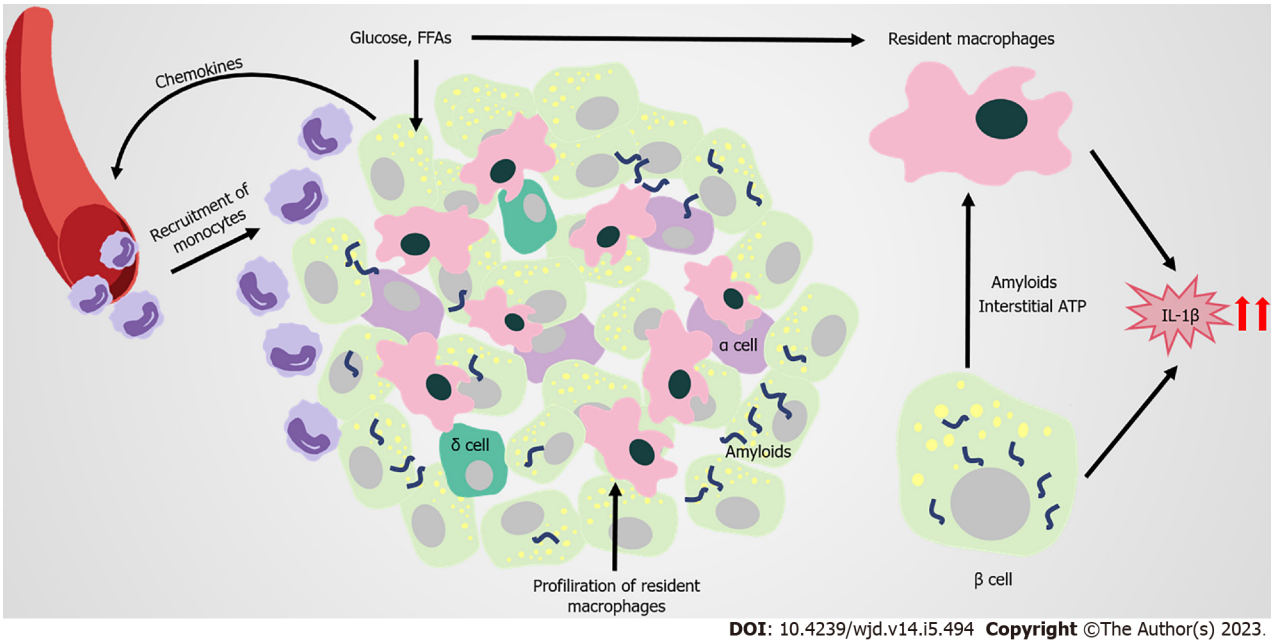

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Figure 4 Immune attack and inflammation in the islet in obesity-related type 2 diabetes. In obesity and type 2 diabetes mellitus (T2DM), the proliferation of islet resident macrophages causes accumulation of macrophages in islets with elevated inflammatory cytokines and chemokines (such as interleukin-1 beta [IL-1β], tumor necrosis factor-alpha [TNF-a]). B cells respond to saturated fatty acids recruit Ly6C+ monocytes to the islets; however, these recruited monocytes remain at the boundary of the exocrine and endocrine pancreas. High concentrations of glucose or free fatty acids and amyloids deposition, promote islet macrophages to produce more IL-1β. Glucose-activated insulin and ATP secretion of β cells also trigger the production of cytokines from macrophages. Elevated IL-1 β levels can promote inflammation in islets, and are closely related to the development of prediabetes and T2DM. FFAs: Free fatty acids.

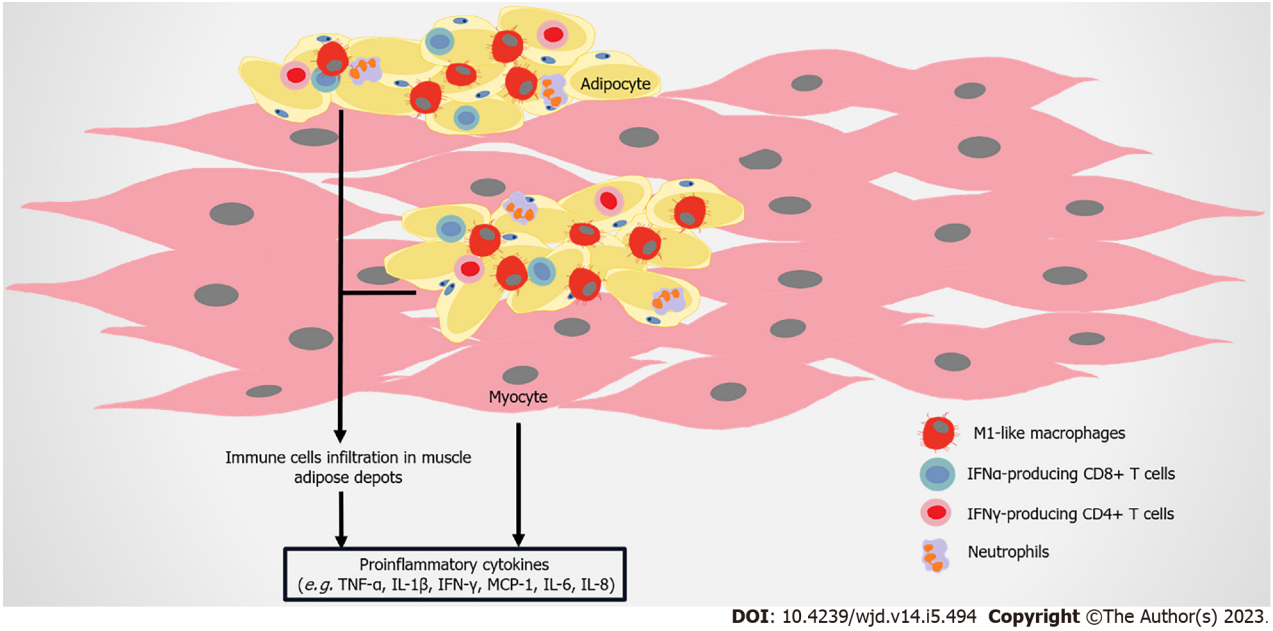

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Figure 5 Immune attack and inflammation in the skeletal muscle in obesity-related type 2 diabetes. As obesity develops, adipose depots between skeletal muscles or surrounding muscles continuously further expand. Immune cells including M1-like macrophages, CD8+ T cells and interferon-gamma (IFN-γ)–producing CD4+ cells, infiltrate into adipose depots in skeletal muscles. Skeletal muscle cells can also produce monocyte chemoattractant protein-1 (MCP1), interleukin-6 (IL-6), IL-8, tumor necrosis factor-alpha (TNF-α), and other molecules, and lead to the infiltration of macrophages, finally inducing insulin resistance.

macrophage infiltration and cytokine response in skeletal muscle, mitigating diet-induced IR[160]. Interestingly, while IL-6 usually promotes inflammation, acute IL-6 treatment in skeletal muscle strengthens insulin-stimulated glucose disposal in humans, possibly mediated by AMP-activated protein kinase signaling[171,172]. Therefore, the exact role of myokines in the metabolism of skeletal muscle needs to be further clarified.

TLRs are also present in skeletal muscle. The expression and signaling of TLR4 is elevated in the muscle of IR patients[173]. LPS-induced IR in skeletal muscle entirely depends on TLR4[174]. The inhibition or deletion of TLR4 prevents acute hyperlipidemia-induced skeletal muscle IR[175,176]. Palmitate induces myeloid differentiation primary response 88 and TLR2 receptor to combine in mouse myotube cells, providing the foundation for inflammation and IR[177]. Therefore, TLRs are also involved in activating proinflammatory factors on skeletal muscle cells.

Overall, many studies support the association of obesity and related-T2DM with increased inflammation of skeletal muscle in rodents and humans. The greater infiltration of macrophages and T cells, and their polarization towards proinflammatory phenotypes, means they act as primary promoters in increasing the inflammation of skeletal muscle. Skeletal muscle cell-secreting myokines also exhibit proinflammatory effects during the development of obesity and T2DM.

CONCLUSION

Chronic low-grade inflammation involving the immune system is a typical feature of obesity-associated T2DM. It generates an inflammatory storm affecting multiple organs and tissues throughout the body. Adaptive activation of the immune system usually stems from an energy imbalance in the body induced by excess calorie intake. However, as the imbalance continues to grow, parenchymal cells and immune cells (in particular, macrophages/monocytes), and their cross-talk, promote the inflammatory response and the development of T2DM by exacerbating IR. Targeting immune cells and relative inflammatory responses is an effective treatment of obesity and associated T2DM.

FOOTNOTES

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REVIEW

Diabetes mellitus and atrial fibrillation-from pathophysiology to treatment

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Abstract

Type 2 diabetes mellitus (T2DM) is a leading risk factor for cardiovascular complications around the globe and one of the most common medical conditions. Atrial fibrillation (AF) is the most common supraventricular arrhythmia, with a rapidly increasing prevalence. T2DM has been closely associated with the risk of AF development, identified as an independent risk factor. Regarding cardiovascular complications, both AF and T2DM have been linked with high mortality. The underlying pathophysiology has not been fully determined yet; however, it is multifactorial, including structural, electrical, and autonomic pathways. Novel therapies include pharmaceutical agents in sodium-glucose cotransporter-2 inhibitors, as well as antiarrhythmic strategies, such as cardioversion and ablation. Of interest, glucose-lowering therapies may affect the prevalence of AF. This review presents the current evidence regarding the connection between the two entities, the pathophysiological pathways that link them, and the therapeutic options that exist.

Key Words: Atrial fibrillation; Diabetes mellitus; Pathophysiology; Treatment

Core Tip: Diabetes mellitus (DM) and atrial fibrillation (AF) are interconnected pathological conditions that are associated with excess morbidity and mortality. DM is implicated in AF's pathophysiology, with mechanisms involving structural remodeling, electrical alterations, autonomic dysfunction, and dysglycemia. The management of this deleterious combination is multifaceted and includes the use of conventional methods such as direct oral anticoagulation, electrical cardioversion, and antiarrhythmic drugs. Sodium-glucose cotransporter-2 inhibitors, catheter ablation, and left atrial appendage occlusion represent appealing modern approaches, whose efficacy in this subgroup of patients needs to be thoroughly examined.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a leading risk factor for cardiovascular complications around the globe and one of the most common medical conditions[1]. Atrial fibrillation (AF) is the most common supraventricular arrhythmia, with a rapidly increasing prevalence[2]. T2DM has been closely associated with the risk of AF development, being identified as an independent risk factor for AF. Furthermore, T2DM has been linked with an increased symptom burden for patients that suffer from AF, leading to impaired life quality and increased hospitalization[3].

The risk of AF development in patients with T2DM has been established by large studies and meta-analyses showing a clear link between AF and T2DM. Based on the association between the two medical conditions and the high risk of cardiovascular morbidity and mortality that their combination presents, literature has concluded that underlying pathophysiology is related to structural, electrical-electromechanical, and autonomic remodeling as well as metabolic parameters[4,5]. Furthermore, their association has highlighted the need for surfacing therapeutic models that can alter the risk of the AF and T2DM combination or lower the risk of AF development in the diabetic population.

In this review, we present the pathophysiologic mechanisms that may combine the two entities, and the therapeutic options that are available for diabetic patients with AF.

AN ASSOCIATION BETWEEN AF AND T2DM

The Women's Health Study established T2DM as a significant predictor of risk for AF[6]. Similarly, a 2010 study suggested a 40% higher risk of developing AF, for diabetic patients, with the overall risk increasing by 3% for every year of T2DM[7]. In 2011, the risk of developing AF in patients with T2DM was identified at 34% over the non-diabetic population[8], while in a 2017 meta-analysis, higher serum glycated hemoglobin levels (HbA1c) were associated with incident AF in prospective cohort studies[9]. In a prospective study, T1DM was associated with a modest increase in the risk of AF in men and a 50% increased risk of AF in women; the risk was proportional to worse glycemic control and renal complications[10]. Similarly, in the prospective cross-sectional observational NOMED-AF study, researchers concluded that AF affects one in four patients with T2DM, highlighting the excessive need for AF screening amongst the diabetic population[11]. Interestingly, a recent Swedish cohort revealed an overall 35% higher risk of AF compared to age- and sex-matched controls from the general population for patients with T2DM; renal complications or poor glycemic control increased the risk of AF[12]. In a Danish study, T2DM was associated with a relative 19% increased risk of incident AF, especially in the 18-39-year-old group[13], while a case-control study concluded that T1DM modestly increases the risk of AF in men but elevates the risk for women by 50%, especially in the cases of poor glycemic control and renal complications[10]. Interestingly, prediabetes, a condition that is also associated with heart failure[14], cardiovascular and all-cause mortality[15], may drive the development of AF[16]. While there is significant evidence pointing concerning the high rates of AF among individuals with T2DM, there is no data on the prevalence of T2DM among AF populations. Thus, the bidirectional relationship between those two entities could only be speculated at present.

The presence of both T2DM and AF can present more complications than each individual entity. In 2022, a meta-analysis of 21 studies concluded that AF patients with T2DM run a higher cardiovascular and all-cause mortality risk[17]. Similarly, in the much earlier ADVANCE study, T2DM patients with AF had an increased risk of major cardiovascular and cerebrovascular events, as well as of cardiovascular and all-cause mortality death, when compared to diabetic patients without AF[18]. Similar results were presented by the ORBIT-AF study, as high symptom burden, low life quality,

cardiovascular and overall mortality were higher for AF patients with T2DM compared to AF patients without T2DM[3]. The 2021 Swiss-AF study also claimed that AF patients with T2DM are less self-aware of AF symptoms and maybe should be systematically screened for silent AF[19]. Moreover, although individuals with T2DM may exhibit a higher thrombotic risk, the rates of electrical cardioversion and catheter ablation use are significantly lower compared to non-T2DM individuals, as shown in the EORP-AF general pilot registry report[1].

PATHOPHYSIOLOGY

Structural remodeling

All pathophysiologic mechanisms are depicted in Table 1 and Figure 1. The most prominent structural modification that AF causes is atrial dilatation and fibrosis. Interestingly, atrial dilatation and fibrosis can result in AF development. In this context, as myocardial fibrosis is independently associated with T2DM, diabetic patients have a prominent substrate for developing AF[4,20]. More specifically, the cellular and molecular underlying mechanisms linking T2DM to myocardial fibrosis include inflammation and oxidative stress deriving from prolonged hyperglycemia[20]. Both increased production of reactive oxygen species (ROS) and decreased expression of enzymes that downregulate ROS have been revealed in diabetic patients, suggesting a high oxidative stress burden[21,22]. A high oxidative stress burden can both result in and aggravate pre-existing inflammation and inflammatory markers such as C-reactive protein and tumor necrosis factor-α, associated with left atrial dilatation and increased AF incidence[23-25]. Furthermore, high levels of ROS result in the activation of fibrotic pathways (*i.e.*, nuclear factor-kappaB pathway) that can result in atrial fibrosis[21].

Furthermore, T2DM upregulates the expression of profibrotic growth factors, such as transforming growth factor (TGF)-β, which activates profibrotic pathways[20,26]. In addition, the increased production of advanced glycation end-products (AGE)s and AGE receptors that derive from T2DM also contributes to atrial fibrosis by upregulating connective tissue growth factors[27]. Fibrosis can slow down atrial conduction and create the substrate for re-entry[28]. Notably, diabetic hearts exhibit enhanced levels of collagen synthesis and high fibroblast activity[29]. We should also mention that the levels of myocardial fibrosis biomarkers, including ST2 and galectin-3, could indicate structural remodeling[25].

In addition, the renin-angiotensin-aldosterone system has also been implicated in promoting fibrosis through the TGF-β signaling pathway[4,20]. Angiotensin II is known to induce cardiac fibrosis[30]. Besides the atria, myocardial fibrosis can also occur in the ventricular myocardium of diabetic patients, resulting in stiffening and diastolic dysfunction of the left ventricle, which is associated with left atrium enlargement[31].

Adiposity may also contribute to atrial interstitial fibrosis and concomitant conduction abnormalities [30]. Obesity is associated with T2DM and lipomatous metaplasia of the heart[31]. In an animal model of a high-caloric diet, authors reported left atrial enlargement, bi-atrial conduction abnormalities, and an increased propensity for inducible and spontaneous AF among the findings[32,33].

Electrical remodeling

Another pathway that may lead to the development of AF in diabetic patients is electrical and electromechanical remodeling. Patients with abnormal glucose metabolism may present conduction abnormalities, such as longer activation times[34]. Experimental data from animal studies suggest that T2DM is linked to abnormal electrical current densities, atrial conduction, and refractory periods, all increasing susceptibility to AF[26,35]. In addition to the electrical and conduction remodeling, T2DM can affect the atrial excitation-contraction coupling, resulting in electromechanical delay (EMD) and arrhythmogenesis, as EMD is an independent predictor of both new and recurrent AF[36,37]. Interestingly, diabetic patients tend to have a higher recurrence of AF after ablation, possibly due to a proarrhythmic substrate caused by electrical remodeling[34]. Furthermore, prolonged conduction times were found in patients with abnormal fasting glucose[38], while EMDs in the atrium are higher in patients with T2DM[37].

Atrial action potential morphology altercations due to ionic currents can alter conduction velocity or susceptibility to triggered activity. In addition, gap junction function may also be affected in the atria of diabetic patients, possibly due to changes in the expression or localization of connexins[30].

Autonomic remodeling

Autonomic dysfunction can also contribute to the development of AF in diabetic patients. Cardiac autonomic neuropathy caused by T2DM contributes to the downsizing of parasympathetic and upregulation of the sympathetic stimuli, resulting in an autonomic imbalance that can excite an arrhythmia, such as AF[39]. A cross-sectional controlled study of 1992 T2DM patients suggested a strong relationship between autonomic dysfunction and silent AF in T2DM originating from autonomic dysfunction[40].

Table 1 Pathophysiologic mechanisms connecting type 2 diabetes mellitus and Atrial Fibrillation

	Involved mechanism	Result
Structural remodelling	Inflammation	Atrial fibrosis and dilatation
	Oxidative stress	
	Expression of profibrotic growth factors	
	Enhanced collagen synthesis and high fibroblast activity	
	Activation of the (RAAS) system	
	Obesity and adiposity	
Electrical remodelling	Longer activation times	Conduction abnormalities
	Abnormal current densities and refractory periods	
	Electromechanical delay	
	Affected gap junction function	
Autonomic dysfunction	Downsizing of parasympathetic nervous system	Autonomic imbalance
	Upregulation of sympathetic stimuli	
Glycemic parameters	Sympathetic activation due to hypoglycaemia	AF susceptibility
	Remodelling due to chronic hyperglycemia	
	Oxidative stress and fibrosis due to glycemic fluctuations	
	Fibrosis due to adipokines	

AF: Atrial fibrillation.

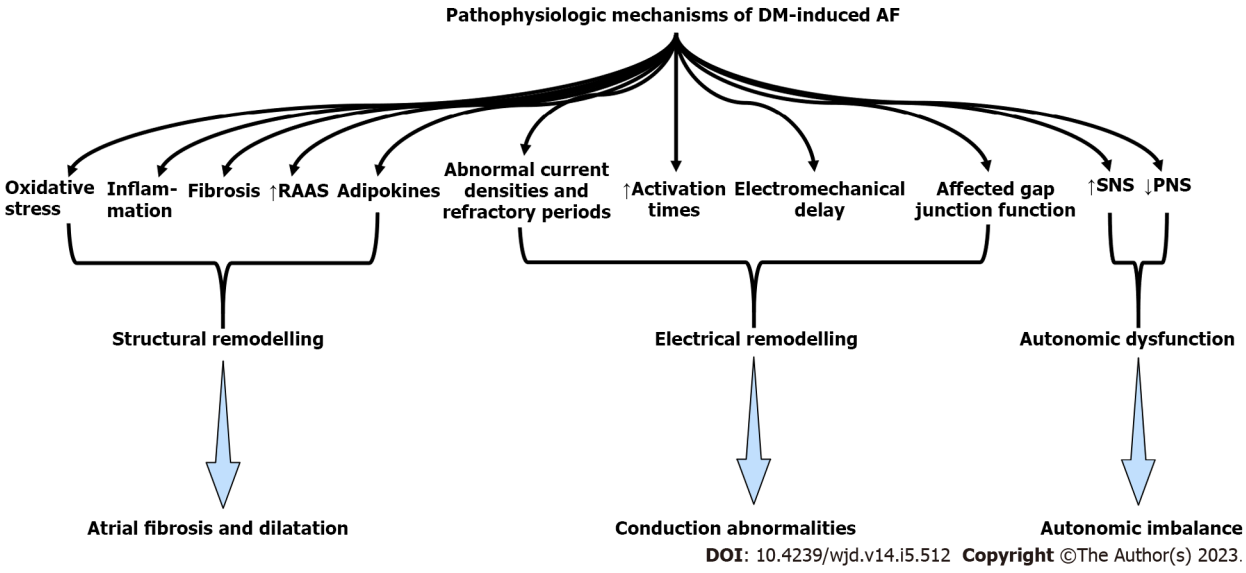

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Figure 1 Pathophysiologic mechanisms of diabetes mellitus-induced atrial fibrillation. DM: Diabetes mellitus; AF: Atrial fibrillation; RAAS: Renin-angiotensin-aldosterone system; SNS: Sympathetic nervous system; PNS: Parasympathetic nervous system.

Glycemic parameters

Patients with T2DM may suffer from hypoglycemia, which can propagate sympathetic activation and overdrive, resulting in an increased risk of AF[41]. The fact that intensive glycemic control does not lower the risk of AF may be attributed to the sympathetic overdrive caused by severe hypoglycemia [42]. On the other hand, chronic hyperglycemia also creates a substrate for atrial remodeling and initiation of AF[4,26]. Hyperglycemia is also associated with enhanced angiotensin II signaling ROS production[43]. Furthermore, high glucose levels can enhance fibrosis through the production of AGEs, which can regulate cardiac fibroblasts by activating their surface receptors[27]. Studies have found, though, that it is actually glycemic fluctuations, rather than chronic hyperglycemia, that may increase

the risk of AF, as they can cause oxidative stress and atrial fibrosis[42,44]. Moreover, a 2017 study revealed that long-term glycemic variability is associated with new-onset AF[45]. It has been suggested that AF and T2DM may share thrombotic pathways. Patients with T2DM suffer from insulin resistance as part of their metabolic profile. In itself, insulin resistance is associated with hypercoagulability, platelet hypersensitivity, endothelial dysfunction, and impaired fibrinolysis, all of which result in high thromboembolic risk[46]. Last, adipokines, signaling modules produced in the epicardial fat layer, have been implicated in the pathophysiology of AF in diabetic patients[30]. Leptin has been found to be associated with atrial fibrosis and AF susceptibility[47]. Other adipokines, such as secreted frizzled-related protein 5, may represent important biomarkers in the risk prediction and management of diabetic complications such as heart failure[48], since they are implicated in mitochondrial energetics, oxidative stress, and apoptosis pathways[49]. However, their role in AF has not been thoroughly assessed. Insulin resistance and adiposity are also considered the main contributors to nonalcoholic fatty liver disease development, a condition that is linked to AF development[50].

TREATMENT

Antidiabetic drugs

Regarding the treatment of diabetic patients, medication should aim to lower blood glucose levels and prevent glycemic fluctuations. Various oral medications are currently being used to treat T2DM, several of which have been associated with a lower risk of AF, as shown in Table 2[4]. Metformin is the most commonly prescribed oral medication. By inhibiting hepatic gluconeogenesis, opposing the action of glucagon, and increasing insulin sensitivity, it exerts its glucose-lowering action. Moreover, its use has been associated with a lower risk for new-onset AF[51]. Several mechanisms have been implicated, including the prevention of the structural and electrical remodeling of left atrium *via* attenuating intracellular ROS, activation of 5′ adenosine monophosphate-activated protein kinase, improvement of calcium homeostasis, attenuation of inflammation, increase in connexin-43 gap junction expression, and restoration of small conductance calcium-activated potassium channels current[52]. Thiazolidinediones (TZD) increase insulin sensitivity by acting on adipose, muscle, and, to a lesser extent, liver to increase glucose utilization and decrease glucose production. Antioxidant effects may be additionally evident, through proliferator-activated receptor-γ agonism and stimulation of catalase[53]. They are also associated with a lower risk of new-onset AF, possibly due to their anti-fibrotic effect[54]; a meta-analysis identified that the risk was reduced by 27% for patients treated with TZDs compared to the control group, especially pioglitazone[55]. On the other hand, sulfonylureas, a widely prescribed second-line hypoglycemic drug category that directly stimulates insulin release from pancreatic beta cells, is not associated with a lower risk for AF[56]. Of interest, sulfonylureas are associated with severe hypoglycemic effects, a substrate for AF development[57]. Insulin therapy has been associated with an increased risk for AF occurrence, possibly due to its hypoglycemic effect[58]. A large study, however, reported no increase in AF incidence with the use of insulin glargine *vs* standard care[59].

Moving to novel antidiabetic agents, dipeptidyl peptidase-4 (DPP-4) inhibitors are glucose-lowering agents that inhibit DPP-4 activity in peripheral plasma, which prevents the inactivation of the incretin hormone glucagon-like peptide-1 (GLP1) in the peripheral circulation. Those agents were found to produce a lower risk of AF when compared to other antidiabetic medications, as shown in a previous study[60]. However, large trials have not revealed a correlation between DPP-4 inhibitors and the incidence of AF[61,62]. Another new class of antidiabetic drugs, GLP1 receptor agonists, are a potent glucose-lowering option by stimulating glucose-dependent insulin release from the pancreatic islets. They exhibit many cardioprotective effects, including antioxidant responses through the upregulation of antioxidant substances (catalase, glutathione peroxidase)[63]. However, they have not been associated with the incidence of AF in large trials; thus, no association between them and AF has been established [64-66].

Sodium-glucose cotransporter-2 (SGLT2) inhibitors lower plasma glucose levels by blocking the reabsorption of filtered glucose at the level of the kidneys. These agents have established cardioprotective effects[67,68], which are dependent on numerous molecular mechanisms, including restoration of beneficial autophagy, antioxidant[63], anti-inflammatory[69,70], and anti-fibrotic responses. SGLT2 inhibitors appear to affect the AF burden. A post-hoc analysis of the DECLARE-TIMI 58 trial reported decreased AF and atrial flutter episodes in individuals with T2DM on dapagliflozin regardless of AF history[71]. Even though the findings from the canagliflozin trial program were neutral[72], recent meta-analyses of randomized controlled trials point to a significant reduction of atrial arrhythmias compared to placebo[73-75]. It also has to be noted that treatment with an SGLT2 inhibitor that was accompanied by a greater than 30% initial decline in the estimated glomerular filtration rate led to a higher risk of AF incidence[76]. In a recently reported Scandinavian cohort study of 79343 new users of SGLT2 inhibitors and 57613 new users of GLP1 receptor agonists, the former was associated with a modestly reduced risk of new-onset AF[77]. Similar findings have also been reported in large registry data analyses[78-80]. Moreover, in elderly individuals with T2DM, the initiation of an SGLT2 inhibitor was accompanied by a lower incidence of AF across the follow-up[81].

Table 2 The effect of antidiabetic medication in atrial fibrillation

Ref.	Medication	Study design	Effect
Chang *et al*[51]	Metformin	Non-RCT	Lower risk of new-onset AF (HR: 0.81, 95%CI: 0.76-0.86, P < 0.0001)
Zhang *et al*[55]	TZD	MA	Approximately 30% lower risk of developing AF compared to controls, only in observational studies
Chang *et al*[60]	DPP4i	Non-RCT	DPP4i users were associated with a lower risk of new-onset AF compared with non-DPP4i
Monami *et al*[66]	GLP1-RA	MA	No effect on AF incidence (OR: 0.87, 95%CI: 0.71-1.05, P = 0.15)
Zelniker *et al*[71]	SGLT2i	RCT	Reduced AF risk (HR: 0.81, 95%CI: 0.68-0.95, P = 0.009)
Fernandes *et al*[73]	SGLT2i	MA	Reduced incidence of atrial arrhythmias (OR: 0.81, 95%CI: 0.69-0.95, P = 0.008)
Engström *et al*[77]	SGLT2i	Non-RCT	SGLT2i modestly reduced AF risk compared to GLP1-RA (adjusted HR: 0.89, 95%CI: 0.81-0.96)
Lee *et al*[80]	SGLT2i	Non-RCT	Lower risk of incident AF compared to DPP4i (HR: 0.68, 95%CI: 0.56, 0.83, P = 0.0001)

AF: Atrial fibrillation; RCT: Randomized controlled trial; HR: Hazard ratio; CI: Confidence interval; TZD: Thiazolidinedione; MA: Meta-analysis; DPP4i: Dipeptyl peptidase 4 inhibitors; GLP1-RA: Glucagon-like peptide-1 receptor agonist; OR: Odds ratio; SGLT2i: Sodium-glucose cotransporter-2 inhibitors.

Experimental studies have been conducted to assess the antiarrhythmic mechanisms of SGLT2 inhibitors. Shao *et al*[82] initially demonstrated the reversal of atrial structural and electrical remodeling induced by T2DM in rats following treatment with empagliflozin. This effect was possibly mediated by the peroxisome proliferator-activated receptor-c coactivator 1α/nuclear respiratory factor-1/mitochondrial transcription factor A signaling pathway[82]. Moreover, the administration of canagliflozin in an experimental model of rapid atrial pacing resulted in a diminished atrial refractory period reduction, suppressed AF inducibility, attenuated atrial interstitial fibrosis, and oxidative stress [83]. A decreased inducibility and duration of pacing-induced AF were also reported in a rat model of mitral regurgitation following treatment with dapagliflozin[84]. Overall, the published preclinical and clinical data regarding the effect of SGLT2 inhibitors on AF appears promising, while appropriately designed randomized controlled trials are warranted to provide further insight into their antiarrhythmogenic potential.

Stroke prevention

Anticoagulants: While AF is independently associated with a high risk of stroke, it seems that DM has an additive effect on the established risk. More specifically, T2DM is associated with a 70% relative increase in the risk of stroke for patients with AF[85]. Of importance, T2DM, as a comorbidity, is included in $CHAD_2DS_2$-VASc risk score, which is the pillar of risk assessment and anticoagulation management[86]. A cohort of 37358 diabetic patients with AF demonstrated that elevated HbA1c levels were associated with an increased risk of stroke[87]. A nationwide cohort study concluded that while in AF patients with T2DM, long-lasting T2DM was associated with a higher risk of thromboembolism, it was not associated with a higher risk of anticoagulant-related bleeding[88]. In addition, the duration of T2DM for over three years was independently associated with a high risk of ischemic stroke for AF patients in the ATRIA study[89]. Insulin-dependent patients exhibit a worse prognosis regarding the incidence of stroke or systemic embolism when compared to diabetic patients who do not require insulin therapy[90]. In an observational cohort, prediabetes was also associated with increased risk for stroke for patients with incident non-valvular AF, even after accounting for other CHA_2DS_2-VASc risk factors[91]. It was also shown that T2DM in AF patients seems to increase the risk of both all-cause and cardiovascular mortality, as well as stroke. Furthermore, HbA1c values of < 6.2% for patients with both conditions predict significantly decreased all-cause and cardiovascular mortality[92].

Based on the $CHAD_2DS_2$-VASc risk score, anticoagulant treatment should be considered in every diabetic patient by default. When contemplating the anticoagulant of choice in this patient population, it has been shown that T2DM affects the time of therapeutic range for AF patients that receive warfarin, a fact that raises safety issues[93]. On the other hand, direct oral anticoagulants (DOACs) use resulted in a 20% reduction in stroke incidents and a 43% reduction in intracranial bleeding compared to warfarin [85]. Furthermore, a study showed that DOACs are as safe and efficient for people with T2DM as for non-diabetic people[94]. A study proposed that dabigatran had the lowest risk for T2DM among AF patients compared to warfarin[95]. For patients with T2DM and CHA_2DS_2-VASc scores ≥ 2, DOACs may be recommended over warfarin[4]. For a CHA_2DS_2-VASc score of 1 in AF patients with T2DM, the optimal type of coagulation has not yet been determined[4]. A 2021 systematic review examining the

safety (hypoglycemia or bleeding) and efficacy (stroke or systemic embolism) of OACs in diabetic patients concluded that DOACs have a better clinical profile than warfarin[96].

Atrial appendage closure: Because of their improved safety and effectiveness profile, DOACs (apixaban, rivaroxaban, dabigatran, edoxaban) have replaced warfarin as the cornerstone of stroke prevention in AF patients. However, alternative treatments must be considered for the subset of individuals at extremely high risk of bleeding. It has long been demonstrated that the great majority (> 90%) of thrombi in nonvalvular AF originate in the left atrial appendage (LAA)[97]. This is a structure of variable form and size with neurohormonal and reservoir functions. Left atrial remodeling with changes in shape, blood flow (stasis), and the presence of trabeculations is thought to be involved in LAA thrombogenesis in AF[98]. T2DM has been associated with adverse LAA remodeling, with important prognostic implications regarding embolic events. Such alterations include the enlargement of the LAA orifice and the reduction of orifice flow velocity, as shown by Yosefy *et al*[99] in a retrospective study of 242 individuals with AF[99]. Interestingly, this appears to be unrelated to the coexistence of AF, as indicated by the experimental study of the same research group[100]. The reduced LAA flow velocity is proportional to the degree of T2DM control, measured by HbA1c[101].

LAA closure (LAAC) is a therapeutic option that is gaining ground in the field of stroke prophylaxis for AF[102]. Surgical LAAC is a technique with confirmed effectiveness, as demonstrated in the recently completed LAAOS-III randomized trial and a recent meta-analysis, for patients with AF who are having cardiac surgery for another cause[103,104]. However, no subgroup analysis according to T2DM status was made, and no safe conclusions can be drawn based on those studies. Percutaneous LAAC has also gained attention recently due to the safety and efficacy of the Watchman and Amplatzer devices, with noninferior outcomes compared to direct OACs in a randomized trial[105]. When examining the devices separately, the landmark trial comparing the Watchman device to warfarin in nonvalvular AF with $CHADS_2$ score ≥ 1 revealed a decreased rate of the primary endpoint (stroke, systemic embolism, and cardiovascular/unexplained mortality) after a 3.8-year follow-up with the device implantation[106]. However, no subgroup analysis based on the presence of T2DM was performed. An upgraded version, the Watchman FLX, is also available and is associated with superior sealing, together with similar safety [107-109], but limited data on the impact of T2DM. Concerning the Amplatzer devices (Cardiac Plug and Amulet), no dedicated large randomized trials are currently available.

The outcomes of LAAC in patients with T2DM have been inconsistent across the reported cohort studies. Litwinowicz *et al*[110] demonstrated similar rates of thromboembolism, mortality, and bleeding events after LAAC between T2DM and non-T2DM individuals[110]. However, in a study of 807 patients undergoing LAAC, T2DM emerged as an independent predictor of the incident early mortality[111]. T2DM was also an independent determinant of hospital readmission 30 and 90 d after LAAC[112]. These T2DM-related readmissions could be more likely associated with gastrointestinal bleeding[113]. Additionally, according to a recent report from the National Cardiovascular Data Registry of 36681 patients receiving the Watchman device, T2DM was an independent variable associated with incident ischemic stroke[114]. To our knowledge, no studies with the Amplatzer devices have assessed the role of T2DM in its safety and efficacy.

Antiarrhythmic strategies

Electrical and pharmacologic cardioversion: T2DM is associated, as comorbidity, with less efficacy of cardioversion. So far, various studies have shown that T2DM results in a lower cardioversion immediate success rate and lower success of sinus rhythm maintenance at 74.5 d follow-up, while it has also been identified as an independent risk factor for cardioversion failure within 30 d[115-117]. Interestingly, T2DM, higher HbA1c, digoxin treatment, and structural and functional cardiac abnormalities were identified as independent risk factors for cardioversion failure and AF recurrence in a 2018 retrospective outcome analysis[117]. In another study, however, this finding was not confirmed[118]. It should also be noted that although spontaneous cardioversion may be seen in a significant proportion of patients with AF, the rates are significantly lower in individuals with coexisting T2DM[119].

Similarly, antiarrhythmic drugs seem less effective for T2DM patients in experimental studies[120], although the evidence is scarce in the clinical setting. Kriz *et al*[121] did not detect a significant association between T2DM and the failure of pharmacologic cardioversion in a single-center study of 236 patients with recent-onset AF[121]. Moving to specific drug classes, in a study of 50 consecutive patients with recent-onset AF, the presence of T2DM did not affect the efficacy of cardioversion with propafenone[122]. Regarding dronedarone use in T2DM, it has been favorably associated with a lower rate of cardiovascular hospitalizations and mortality, as well as greater freedom from AF, compared to placebo[123]. At the same time, no data are available for the specific subgroup of AF patients with T2DM who receive amiodarone. However, a previous study has suggested a delayed antiarrhythmic effect of amiodarone in individuals with T2DM, partly attributed to diabetic autonomic neuropathy [124]. Often, due to concomitant QTc prolongation, silent coronary artery disease, or renal failure, patients with T2DM may be at higher risk of developing adverse effects from antiarrhythmic drug therapy[62,125]. Despite that, a study by D'Angelo *et al*[126] observed that patients with T2DM were less likely to discontinue the prescribed antiarrhythmic regimen[126].

Ablation: Regardless of symptoms, early rhythm management is critical in lowering the burden of AF consequences[127,128]. Percutaneous catheter AF ablation is an appealing technique for rhythm regulation. The most often used ablation treatment in electrophysiology is radiofrequency catheter ablation. It mainly consists of pulmonary vein isolation, which is thought to be a key trigger of paroxysmal AF[129]. Catheter ablation is a well-established treatment for drug-refractory, symptomatic AF with a variety of clinical benefits and better AF control for diabetic patients when compared to antiarrhythmic drugs[130]. Despite that fact, individuals with T2DM may be less likely to receive catheter ablation, as pointed out by the recent study of Quiroz *et al*[131]. However, the rate of T2DM patients receiving this treatment has increased over the years[132].

There have been reports of a lower efficacy of catheter ablation in individuals with T2DM than in those without T2DM. This could be due to the fact that the induced scar may impair atrial relaxation, promoting a stiff left atrial phenotype in individuals with T2DM[133]. Wang *et al*[134] highlighted that T2DM was associated with lower arrhythmia-free intervals in patients with T2DM after a median 29.5-mo follow-up[134]. A recent study of 369 patients with AF reported that T2DM was a predictor of AF recurrence in patients with paroxysmal AF[135]. This has not been the case in persistent AF, where the already established fibrotic changes may account for the increased risk of recurrence[136]. The performance of a second-generation, cryoballoon-based procedure may be accompanied by similar success rates in T2DM and non-T2DM patients, as pointed out by the study of Amr *et al*[137]. Moreover, T2DM is among the variables of the DR-FLASH score that has been utilized to identify individuals with a greater burden of arrhythmogenic substrates that may benefit from extensive ablation beyond the pulmonary veins[138,139]. T2DM is also an independent predictor of pulmonary vein stenosis after catheter ablation, as shown by the ADVICE trial[140]. It should also be mentioned that individuals with T2DM may be less likely to receive catheter ablation, as pointed out by the recent study of Quiroz *et al*[131]. However, the rate of T2DM patients receiving this treatment has increased over the years[132].

Other studies have concluded that there is no difference in post-ablation recurrence between diabetic and non-diabetic patients[141,142]. The degree of glycemic control might be an important confounding variable. More specifically, a 2015 metanalysis concluded that AF ablation has similar safety and efficacy for diabetic patients as for the general population, especially for younger patients with efficient glycemic control; however, it was shown that higher basal glycated hemoglobin levels were associated with a higher incidence of AF recurrence after catheter ablation[143]. Although the literature has not yet concluded, insufficiently managed T2DM may be a risk factor for AF recurrence following catheter ablation[144]. T2DM has also been correlated with a higher risk of cardioversion failure for early AF recurrence (≤ 7 d) after ablation[115].

Antidiabetic drugs may alter the efficacy of AF ablation in individuals with T2DM. Metformin was recently shown to be independently associated with a lower risk of AF recurrence in T2DM patients after catheter ablation[145]. A randomized trial contemplating the effect of SGLT2 inhibitors on AF following ablation concluded that tofogliflozin exhibited a better profile and less AF recurrence when compared to anagliptin[146]. Previously, dapagliflozin was an independent predictor of longer arrhythmia-free intervals in patients with T2DM undergoing radiofrequency catheter ablation after a mean follow-up of 15.5 mo[147].

CONCLUSION

DM and AF are widely affiliated entities. DM has been closely associated with the risk of AF development, identified as an independent risk factor for AF. Regarding cardiovascular risk and mortality, the presence of both conditions has been linked with high mortality. Even though the pathophysiology is still not fully determined, structural, electrical, and autonomic pathways have been identified as underlying mechanisms. Regarding therapy, novel antidiabetic agents and revolutionary antiarrhythmic and antithrombotic strategies are being examined concerning the optimal therapeutic plan for diabetic patients with AF.

FOOTNOTES

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MINIREVIEWS

What, why and how to monitor blood glucose in critically ill patients

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Abstract

Critically ill patients are prone to high glycemic variations irrespective of their diabetes status. This mandates frequent blood glucose (BG) monitoring and regulation of insulin therapy. Even though the most commonly employed capillary BG monitoring is convenient and rapid, it is inaccurate and prone to high bias, overestimating BG levels in critically ill patients. The targets for BG levels have also varied in the past few years ranging from tight glucose control to a more liberal approach. Each of these has its own fallacies, while tight control increases risk of hypoglycemia, liberal BG targets make the patients prone to hyperglycemia. Moreover, the recent evidence suggests that BG indices, such as glycemic variability and time in target range, may also affect patient outcomes. In this review, we highlight the nuances associated with BG monitoring, including the various indices required to be monitored, BG targets and recent advances in BG monitoring in critically ill patients.

Key Words: Blood glucose; Continuous glucose monitoring; Critical care; Glycaemic indices; Hypoglycaemia; Intensive care unit

Core Tip: Blood glucose (BG) monitoring is a vital component of critical care management. Even non-diabetic critically ill patients are prone to glycemic fluctuations necessitating frequent blood sampling and BG monitoring. Multiple medications, presence of underlying comorbidities and organ dysfunctions, and rapidly changing patient condition make BG control challenging in critically ill patients. Even the commonly used capillary blood sampling for BG monitoring may not be reliable in these patients. In addition to the established parameters of hypoglycemia and hyperglycemia, newer glycemic indices like glycemic variability and time in target range have also been recognized to affect outcomes of critically ill patients, further complicating BG monitoring. Devices for continuous glucose monitoring are also being increasingly tested in these patients, and their use in conjunction with artificial intelligence-based devices may provide a solution to comprehensive glucose control in the future.

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INTRODUCTION

Blood glucose (BG) monitoring is a vital component of critical care management. Diabetes is an important risk factor for developing severe disease necessitating intensive care unit (ICU) admission. Additionally, any acute illness may increase the risk of derangement of BG levels. These fluctuations may happen irrespective of the diabetes status of the patient and may affect their ICU course and outcomes. Several factors have been identified that increase the risk of developing hyperglycemia and hypoglycemia in ICU patients (Table 1)[1-5]. The use of multiple medications, underlying comorbidities and organ dysfunctions, and rapidly changing patient conditions make BG control challenging in critically ill patients. Even the commonly used capillary blood sampling for BG monitoring may be unreliable in these patients[6].

Furthermore, glycemic indices and targets for optimizing outcomes in critically ill patients need to be clarified. Targeting tight glucose control, which was earlier recommended, has not shown any mortality benefit but may increase the risk of hypoglycemia by five times[7]. It also requires frequent blood sampling and regulation of insulin dose, which may increase the workload of healthcare workers and add to the cost of care. Hence, recent guidelines recommend more liberal BG targets to avoid the risk of hypoglycemia[8,9]. In addition to the commonly employed indices such as hyperglycemia and hypoglycemia, glycemic variability (GV) and time in target range (TITR) are recently recognized components of dysglycemia which may affect patient outcomes[10-12]. However, the exact targets for these indices still need to be well established.

ARTERIAL VS CAPILLARY MONITORING

BG management requires frequent blood sampling and insulin dose adjustment. BG monitoring in critically ill patients by plasma-based central laboratory methods using venous or arterial samples is considered standard. However, due to the long turnaround time and convenience associated with a point of care testing (POCT), currently, glucometers and arterial blood gas (ABG) analyses are being frequently used. Bedside capillary blood glucose monitoring arguably remains the most commonly employed method, even in critically ill patients. However, its accuracy may be affected in patients with subcutaneous oedema, shock, and hypoxemia, which commonly affect ICU patients[4]. This may lead to highly variable results and higher bias (overestimation) for fingerstick sampling than arterial or venous BG monitoring, which can significantly affect clinical decision-making[13]. Hence, arterial blood is preferred but requires repeated arterial punctures or an invasive arterial line (Table 2). The correlation between arterial and capillary glucose levels is also significantly affected in patients with shock requiring vasopressors, with a proportion of disagreement ranging from 1.4% to 27.1%[14,15].

Over the years, there has been remarkable progress in the technologies used for bedside glucometers. Based on the glucose oxidase method, the initial generation of glucometers was affected by low and high haematocrit, blood pH, and even some medications[16]. The more recent glucose dehydrogenase-based glucometers are largely unaffected by high PaO_2 and other interferences but had a serious flaw of being highly inaccurate in patients on peritoneal dialysis whose dialysate contains Icodextrin, because of its hydrolysis to maltose, causing pseudo-hyperglycemia[17]. The accuracy and precision of the newer generation of glucometers have improved significantly. They have largely overcome the fallacies of their predecessors to acceptable clinical levels, especially if arterial or venous blood is used for

Table 1 Risk factors for developing hyperglycemia and hypoglycemia in intensive care unit patients

Risk factors for hyperglycemia	Risk factors for hypoglycemia
Release of stress hormones: Corticosteroids and catecholamines	Targeting tight glucose control with insulin infusions
Release of proinflammatory mediators	Use of bicarbonate-containing fluids
Administration of exogenous drugs: Corticosteroids, vasopressors, ascorbic acid	Interruption of nutritional support
Parenteral solutions containing dextrose	Infection, sepsis
Stress-induced hyperglycaemia	Drugs *e.g.* Octreotide, anti-glycaemic agents, betablockers, antibiotics (levofloxacin, quinine, trimethoprim-sulfamethoxazole)
Use of commercial dietary feeds or supplements	Use of vasopressors
	Liver failure
	Dialysis support

Table 2 Comparison between arterial and capillary monitoring of glucose

	Arterial	Capillary
Accuracy	As accurate as laboratory testing	Accuracy affected by poor perfusion states, pH, anaemia, renal failure, and high oxygen tension levels (old generation glucose oxidase based glucometers)
		Overestimation in all glucose range, especially in hypoglycaemic range
Sample volume	0.25-1 mL (can be more depends on method)	Minimal
Other variables	Simultaneous measurement of electrolytes, haemoglobin, and blood gases (partial pressure of oxygen and carbon dioxide, pH)	Single variable measured is sugar
Pain	Arterial sampling required	Repeated pin prick may cause patient discomfort
	Convenient in patients with indwelling arterial line	
Need of expertise	Needs arterial line or arterial sampling which needs expertise	Simple finger stick, no expertise needed

analysis. Recent data suggest that these devices may achieve more than 97% correlation with the reference standard when testing venous and arterial samples. These systems have demonstrated acceptable clinical performance with high specificity, sensitivity, and low risk of potential insulin-dosing errors[18].

It can be inferred that arterial blood should be preferred over capillary blood for glucose monitoring, irrespective of the method used, provided standards of calibration are being followed. Although capillary glucose serves well in hospitalized patients, caution should be exercised in patients with shock [14], insulin infusion[15], on vasopressors[14,19], coma[20], and other critically ill adult patients[6]. A large meta-analysis with 21 studies showed that BG readings taken from arterial samples were significantly more accurate than those taken from the capillary samples. Again, as compared to glucometer readings, readings taken from ABG analyzers were more accurate, especially in the hypoglycemic range[6]. Despite venous samples tested in the laboratory remain the gold standard, POCT using arterial samples analyzed using ABG analyzers may provide an accurate estimation of the BG levels with the advantage of rapid turnaround time and may provide more clinically relevant and actionable information.

CONTINUOUS GLUCOSE MONITORING

Continuous glucose monitoring (CGM) devices have evolved from retrospective analyzers validated in outpatient services and can now be utilized in hospitalized patients to optimize glucose control. These devices have been associated with better control of short-time fluctuations in BG levels, reduced glycated hemoglobin (HbA1c) values, reduced risk of severe hypoglycemia, improved glycemic control, increased treatment satisfaction, and may also reduce healthcare costs[21,22]. Numerous CGM devices are commercially available, which are approved for in-hospital use. These devices are classified as non-

invasive (transdermal), minimally invasive (subcutaneous) and invasive (intra-vascular).

The real-time analyzers have a subcutaneous cannula with a biosensor to analyze glucose from interstitial fluid, which is then relayed wirelessly by the attached transmitter to the monitors[23]. Even though the initial trials with CGM devices showed a reduction in hypoglycemic events as compared to the intensive insulin protocols measuring glucose samples frequently, these devices failed to reduce the GV[24,25].

The newer systems have shown a fair correlation in direct comparison with each other and capillary measurements in non-critically ill diabetic patients[26]. However, the data from critically ill patients, was lacking so far. Early results from testing in critically ill patients with coronavirus disease 2019 (COVID-19) have been encouraging, and these devices have been shown to have good accuracy, increase TITR, and reduce GV[27,28]. The latest generation of continuous subcutaneous flash glucose monitoring system (FreeStyle Libre) has been shown to have high test-retest reliability and acceptable accuracy even in critically ill patients[29,30].

Although evidence is still evolving, some drawbacks exist (Table 3). There is usually a time lag between blood and interstitial fluid to equilibrate, which hinders accurate real-time sampling[31]. Other issues which are worth considering are variable biosensor life, need for frequent calibration, and limited working range (BG levels between 40 and 400 mg/dL). Their efficacy has still not been evaluated in patients with severe oedema due to hypoalbuminemia and hepatic failure, in whom the correlation between blood and interstitial fluid might be altered and inaccurate[23]. Additionally, the presence of hypoxemia and shock may also affect their accuracy.

These shortcomings can be overcome by using intravenous CGM systems, which are more accurate, making frequent monitoring possible in critical patients without putting extra-time load on nursing staff. In addition, these devices can also be integrated with closed-loop systems providing an automated insulin delivery to improve BG management[32]. However, their application is also associated with a high incidence of sensor failure, loss of venous integrity, and logistic issues[33]. In addition, finding a suitable vein may also be an issue in critically ill patients[34].

The evidence supporting the clinical effectiveness and efficiency of these systems in ICU patients is still limited. Their impact on clinically relevant outcomes like ICU mortality, length of stay (LOS) in hospital and ICU remains unknown[35]. Moreover, validation of these systems in various ICU populations may lead to their widespread use, considering the advantages of avoiding hypoglycemia, hyperglycemia, and GV and reducing nursing loads with less need for finger pricks. Even though these devices may not be beneficial to all critically ill patients, they may benefit some specific ICU patients such as those on intravenous insulin or corticosteroids, and patients with end-stage organ dysfunction (renal or liver), post-operative neurosurgery or those with traumatic brain injury and post-organ transplant[36-38]. CGM is effective and safe in critically ill COVID-19 patients and may significantly reduce the need for bedside BG testing; thus, it is recommended to use CGM in these patients to reduce nursing exposure[39].

GLYCAEMIC INDICES

Traditionally glycemic control has been defined as highest and lowest target BG levels with an aim to prevent episodes of hypoglycemia and hyperglycemia. In recent years, studies have evaluated other aspects to dysglycemia and their association with clinical outcomes in critically ill patients. Variability of these indices is a predictor of worse patient outcomes, independent of frequency and severity of hypoglycemia and hyperglycemia[40,41]. Even though the current glycemic management guidelines do not recommend any specific target for many of these indices, based on the current data some suggestions may be made to optimize glycemic control in critically ill patients (Table 4)[8,41-45].

BG targets

Safe BG levels have been challenging to define in critically ill patients. Till recent years glucose control in ICUs has swayed between tight glycemic control (avoiding hyperglycemia) to liberal glucose control (avoiding hypoglycemia) in different case mix populations[46,47].

The American Diabetes Association recommends that a BG level below 180 mg/dL is acceptable for ICU patients[8]. In patients with sepsis, the recent version of surviving sepsis guidelines recommend targeting BG levels between 140 and 180 mg/dL and initiating intravenous insulin therapy if BG levels are above 180 mg/dL for two consecutive readings[9]. They further recommend measuring BG levels every 1-2 h, especially in the first 24 h after admission.

GV

The GV can be defined as the measurement of fluctuations of BG over a given interval of variable time. Markers of GV like standard deviation, coefficient of variation, mean amplitude of glycemic excursion, and one time-weighted index, the glycemic lability index (GLI), are significantly associated with higher risk of infections and mortality in medical-surgical ICU patients, even though the mean BG failed to show any association. Additionally, the patients in the upper quartile of GLI had the strongest

Table 3 Advantages and disadvantages of continuous glucose monitoring

Advantages	Disadvantages
Real-time interstitial glucose	Lag time of 15 min from blood glucose, in transdermal and subcutaneous devices (Caution if levels are fluctuating rapidly)
Deviation from arterial blood glucose is less than 20%	Direct vascular sampling continuous monitoring devices are still evolving
Provides long-term day-to-week blood glucose levels	Frequent calibration (2-3 times per day)
Reduced hypoglycaemic events	Biosensors have limited life (around 7 d)
Less labour intensive	Limited glycaemic range 40-400 mg/dL
Can reduce contact of care-givers reducing cross infections and risk to care-givers	Evolving clinical evidence (especially in critically ill patients)
	Invasive device, risk of infection when using intravenous devices

Table 4 Suggested targets for various glycemic indices in critically ill patients

Glycemic indices	Suggested targets
Blood glucose	140-180 mg/dL
Time in range	More than 70%
Glycaemic gap	Less than 25.89 mg/dL in type 2 diabetics
	Less than 40 mg/dL in community acquired pneumonia
Glycaemic lability	Below median (40 mmol/L^2/h/week)
Stress hyperglycaemia ratio	Less than 1.14 in sepsis patients
Mean amplitude of glycaemic excursions	Less than 65 mg/dl in sepsis patients
Coefficient of variation	Less than 36%

association with infections [odds ratio (OR): 5.044, P = 0.004][41]. Even after correcting for hypoglycemia, GV has been reported to be an independent predictor of worse patient outcomes. In fact, GV has been shown to be a precursor of hypoglycemia, as the risk of hypoglycemia is 3.2 times higher in patients with increased GV[48].

TITR

TITR is the percentage of time where the BG stays in the pre-defined glycemic range, calculated per patient per day and expressed as a percentage of time spent. Glucontrol was one of the earliest randomized control trials (RCT) to show that TITR above 50% was independently associated with improved survival rates in critically ill patients irrespective of whether tight (80–110 mg/dL) or liberal (140–180 mg/dL) glycemic control was applied[49].

In another study, when three thresholds of TITR of 30%, 50%, and 70% were compared in 784 medical surgical patients, it was reported that there was significantly reduced organ failure with TITR of 50%. Additionally, a TITR above 70% further resulted in significantly improved survival rates[42]. Similarly, improved outcomes in terms of reduced sternal wound infection and LOS on invasive mechanical ventilation (IMV) and in ICU has been reported in cardiac surgery patients who could achieve TITR above 80%[22]. The exact cut-offs remain to be defined as different studies have suggested TITR from 50%-80% to improve patient outcomes[22,42].

Glycemic gap

Glycemic gap is calculated by subtracting HbA1C-derived average glucose = [(28.7 × HbA1c) - 46.7] from plasma glucose at admission. In a cohort of 200 patients with type 2 diabetes mellitus admitted to ICUs, the glycemic gap was found to be a predictor of multi-organ dysfunction syndrome (MODS), acute respiratory distress syndrome, shock, upper gastrointestinal bleeding, and acute renal failure (ARF). A glycemic gap of 25.89 mg/dL was predictive for the combined occurrence of mortality, MODS, and ARF[43]. Similarly, in a retrospective analysis of patients with community-acquired pneumonia, an elevated glycemic gap of 40 mg/dL had an OR of 3.84 for the incidence of a composite of adverse outcomes, which included length of IMV, and LOS in the ICU and hospital[50].

Glycemic lability

A glycemic lability (GL) is a measure of GV which records the change in glucose level over weeks

calculated from all recorded glucose values. In a multicentric study, where GL and time-weighted average BG were calculated and analyzed, compared to patients with GLI below median 40 (mmol/L^2/h/week), patients with GLI above this median had a significantly longer ICU stay and a higher ICU and hospital mortality. There was no significant association between GLI and mortality when comparing patients with and without diabetes and baseline HbA1c values. It was found that high GV, as determined by the GLI, was associated with increased hospital mortality independent of average BG, age, diabetes status, HbA1c, hypoglycemia, and illness severity[44].

Stress hyperglycemia ratio

Stress hyperglycemia ratio (SHR) is defined as the ratio of plasma glucose to average glucose derived by HbA1C [(1.59 × HbA1c) – 2.59], where HbA1c is used to estimate average glucose concentration over the prior three months. It accounts for acute stress-induced hyperglycemia and long-standing glycemic control. GLI and SHR are indices which account for premorbid glycemic control. Preliminary reports suggest that SHR may be a better marker of patient outcomes than hyperglycemia[51]. In specific patient populations, SHR has been shown to be a predictor of hemorrhagic conversion in acute ischemic stroke and poor outcomes in acute coronary syndrome[52,53]. In diabetic patients with sepsis, a high SHR (≥ 1.14) has been shown to be predictive of mortality[45]. While the exact cut-off value for SHR remains unclear, different SHR definitions have been used in the literature[54].

SHR1 = fasting glucose (mmol/L)/glycated haemoglobin (HbA1c) (%)

SHR2 = fasting glucose (mmol/L)/[(1.59 × HbA1c) - 2.59]

SHR3 = admission BG (mmol/L)/[(1.59 × HbA1c) - 2.59]

SHR1 and SHR2 have been shown to be independently associated with worse clinical outcomes in patients with ischemic stroke after intravenous thrombolysis. Furthermore, SHR1 has been shown to have a better predictive performance for outcomes as compared to other SHR definitions[54].

Diabetic status and glycemic targets

The effect of acute and chronic hyperglycemia on modifying glycemic targets to optimize glycemic control in critically ill patients is yet to be studied in detail. The results from a study by Krinsley and Preiser[55] suggested that TITR greater than 80% for a BG target between 70 and 140 mg/dL was strongly associated with increased survival in critically ill patients without diabetes mellitus. However, such a relationship was not found in diabetic patients[55]. Lanspa *et al*[56] also reported that a TITR greater than 80% was associated with reduced mortality in non-diabetic patients and in those with well-controlled premorbid diabetes (judged by admission HbA1c). However, no such association could be shown in patients with poorly controlled diabetes[56].

In another study, a lower hospital mortality rate was observed in patients with higher (> 7%) preadmission levels of HbA1c and higher time-weighted average glucose concentration in critically ill patients. This suggests that patients with chronic hyperglycemia may benefit from more liberal glucose control and may tolerate a higher BG level[57]. However, such claims need to be better evaluated in large-scale trials before they are applied in routine clinical practice.

ROLE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence (AI)-based applications and devices have been in clinical use to manage non-critically ill diabetic patients for a long time. These devices have been used in patient-centered care to make an early diagnosis, predict complications, and even engage patients to ensure treatment adherence. There has been a heightened interest in AI applications for critically ill patients in the last few years. Even though there is insufficient evidence for its routine use, AI is increasingly utilized and can potentially change the future of critical care glucose management (Table 5)[58].

In ICU, frequent blood sampling and insulin dose adjustments are required to maintain glycemic control, increasing nursing workload and chances of error. AI has the potential to improve glycemic control while reducing nursing workload and errors. The LOGIC-1 and LOGIC-2 RCTs showed that software-guided algorithms could achieve better glycemic control than nurse-guided protocols without increasing the risk of hypoglycemia[59,60].

AI-based insulin bolus calculators and advisory systems like MD-Logic controllers are commercially available and have been shown to provide better glycemic control and reduce nocturnal hypoglycemic events[61]. Software-based algorithms have been used to regulate insulin infusion based on the patient's glucose levels. Model predictive controls use algorithms based on patient parameters like their age and diabetes status, along with the dose of dextrose administered and the insulin sensitivity, which can predict the patient's response to hyperglycemia and insulin therapy and adjust the insulin dose accordingly. These algorithms can improve the accuracy of predicting hyperglycemia, reduce the need for repeated blood sampling, and provide highly individualized insulin therapy[62,63].

CGM devices (Dexcom G6™) have been integrated with automated insulin suspension using AI algorithms (Basal-IQ™ technology). AI-based algorithms can predict when the BG levels may fall below the predefined levels and can alter the insulin infusion accordingly[64]. These CGM regulated insulin

Table 5 Possible critical care applications of artificial intelligence in diabetes management

Potential applications	Clinical examples
Blood glucose monitoring and prediction of adverse glycaemic events	Early detection of hypoglycaemia and hyperglycaemias *e.g.*, MD-Logic controller
Blood glucose control strategies	Software-based algorithms for insulin dosing *e.g.*, proportional-integral-derivative models, Glucose Regulation for Intensive Care Patients, and Model predictive controls
Insulin bolus calculators and advisory systems	CGM regulated insulin infusion system predicting hypoglycaemia and regulating insulin doses
	Artificial intelligence based artificial pancreas
Risk and patient stratification	Prediction of sepsis and risk of nosocomial infections
	Risk of renal and cardiac complications like acute kidney injury and myocardial infarction
	Need for ICU admission
	ICU mortality

CGM: Continuous glucose monitoring, ICU: Intensive care unit.

infusion systems have been shown to reduce the episodes of hypoglycemia effectively[65].

AI-based artificial pancreas (AP) has been shown to provide comprehensive glycemic control by effectively controlling BG levels, reducing wide glucose excursions, reducing episodes of hypoglycemia and hyperglycemia, and increasing the percentage of TITR. Even in critically ill patients, AP achieved stable glucose control and reduced GV while reducing the episodes of hypoglycemia or hyperglycemia and the need for frequent sampling, thereby reducing the nursing workload[66-68]. Whether the use of AP can improve clinical outcomes and has a favorable cost-benefit ratio, still needs to be evaluated.

In addition to predicting long-term or chronic complications, AI may also be instrumental in predicting acute life-threatening complications like acute myocardial infarction in patients with diabetes [69]. AI using a convolutional neural network has been shown to be highly accurate in predicting mortality in critically ill diabetes patients with an area under the curve of 0.97[70,71]. However, these models need to be compared to more widely used and validated models for mortality prediction in ICU patients.

AI applications may improve patient care and outcomes and improve glycemic control while reducing nursing workload. As AI-based devices may enable us to monitor and institute therapy remotely, they may be particularly useful in managing highly infectious diseases like COVID-19. However, AI is still in the early stages of development and AI-based applications still need to be thoroughly evaluated and validated in critically ill patients. In addition, the need for more regulations, recommendations, and guidelines for using AI limit its applicability. Safety, liability, and reliability issues pertaining to AI application need to be better assessed before it is integrated into the existing healthcare infrastructure and becomes acceptable at a larger scale.

CONCLUSION

ICU patients are a unique population with dynamic clinical conditions and therapeutic needs. High physiological stress, raised inflammatory cytokines, varying nutritional intake, and fluctuating organ functions make glycemic control challenging in these patients. Guidelines may aid us in providing a generalized approach to glycemic control, but there may be a need for a more personalized approach to reducing the harmful effects of dysglycemia. The newer glycemic indices like GV and TITR may allow us to achieve patient-centered care with better glycemic control. However, their exact targets and impact on patient outcomes need to be better evaluated before they are routinely recommended. The use of AI-based applications may provide a more comprehensive solution in the future, but presently close monitoring and early detection and management of complications constitute the mainstay of glucose management.

FOOTNOTES

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MINIREVIEWS

Exercise interventions for patients with type 1 diabetes mellitus: A narrative review with practical recommendations

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Abstract

Type 1 diabetes mellitus (T1DM) is a chronic endocrine disease that results from autoimmune destruction of pancreatic insulin-producing β cells, which can lead to microvascular (*e.g.*, retinopathy, neuropathy, and nephropathy) and macrovascular complications (*e.g.*, coronary arterial disease, peripheral artery disease, stroke, and heart failure) as a consequence of chronic hyperglycemia. Despite the widely available and compelling evidence that regular exercise is an efficient strategy to prevent cardiovascular disease and to improve functional capacity and psychological well-being in people with T1DM, over 60% of individuals with T1DM do not exercise regularly. It is, therefore, crucial to devise approaches to motivate patients with T1DM to exercise, to adhere to a training program, and to inform them of its specific characteristics (*e.g.*, exercise mode, intensity, volume, and frequency). Moreover, given the metabolic alterations that occur during acute bouts of exercise in T1DM patients, exercise prescription in this population should be carefully analyzed to maximize its benefits and to reduce its potential risks.

Key Words: Type 1 diabetes mellitus; Exercise; Resistance training; High-intensity interval training; Aerobic training; Quality of life

Core Tip: Our manuscript analyzed the benefits of physical exercise for patients with type 1 diabetes mellitus. Benefits of different types of physical exercise (*e.g.*, aerobic training, resistance training, and high-intensity interval training) and the possibilities of application for each were analyzed. We discussed the level of physical and physiological fitness as well as the implications of exercise on quality of life, quality of sleep, enjoyment of exercise, and motivation towards physical exercise. Finally, a practical proposal of a physical exercise program for patients with type 1 diabetes mellitus was created.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease that results from the immunological destruction of pancreatic insulin-producing β cells, which can lead to microvascular (*e.g.*, retinopathy, neuropathy, and nephropathy) and macrovascular complications (*e.g.*, coronary arterial disease, peripheral artery disease, stroke, and heart failure) as a consequence of chronic hyperglycemia[1]. According to the International Diabetes Federation and World Health Organization, 25-45 million adults (> 20-years-old) suffer from T1DM worldwide[2]. In addition, it is estimated that the number of people with T1DM in the world will increase 25% by 2030[3].

Despite the widely available and compelling evidence that regular exercise is an efficient strategy to prevent cardiovascular disease and to improve functional capacity and psychological well-being in people with T1DM, over 60% of individuals with T1DM do not exercise regularly[4,5]. Lack of time, fear of a hypoglycemic event, and loss of glycemic control due to inadequate knowledge of exercise variable management are the main barriers to increasing physical activity in patients with T1DM[6]. It is, therefore, crucial to devise approaches to motivate patients with T1DM to exercise, to adhere to a training program, and to inform them of the specific characteristics of the training program (*e.g.*, exercise mode, intensity, volume, frequency). Moreover, given the metabolic alterations that occur during acute bouts of exercise in T1DM patients, exercise prescription in this population should be carefully analyzed to maximize benefits and to reduce potential risks.

AEROBIC EXERCISE AND T1DM

Aerobic exercise guidelines and benefits

Aerobic exercise is defined as continuous physical exercise of moderate intensity (50%-70% of maximum heart rate) and of high volume (> 20-30 min), which involves large muscles and requires the presence of oxygen to obtain energy[7]. Examples of this exercise mode are cycling, swimming, walking, or running performed at a moderate intensity[7]. This type of exercise has traditionally been recommended for specific populations, such as T1DM. In fact, the American Diabetes Society recommends at least 150 min per week of aerobic exercise for better glycemic regulation and improvement of the disease[8].

Aerobic exercise has positive effects on T1DM patient health, improving insulin sensitivity, body composition, endothelial, pulmonary, and cardiac function, as well as cardiorespiratory fitness[7] (Figure 1). It is obvious that aerobic exercise training may robustly protect people with T1DM from several complications associated with cardiovascular disease, the main cause of mortality and morbidity in this population[9].

Aerobic exercise in T1DM population: General considerations

T1DM patients must consider various factors before performing continuous moderate-intensity exercise safely. Before starting the training program, certain factors must be considered. The patient's physical condition level/capacity, previous exercise experience, the duration and intensity of the current exercise, blood glucose at that given moment, the dose of pre-exercise administered insulin, and finally the general diet in the preceding period[4,10]. Exogenously administered insulin allows glucose to enter into muscle cells, consequently generating the energy to maintain movement since the entire metabolism during and after any given exercise will be altered.

During aerobic exercise, blood glucose enters the muscles to meet the needs for increased energy generation in the presence of oxygen initiating aerobic glycolysis. Physical exercise can increase muscle glucose demand and consumption up to 50-fold through an increase in insulin sensitivity and an

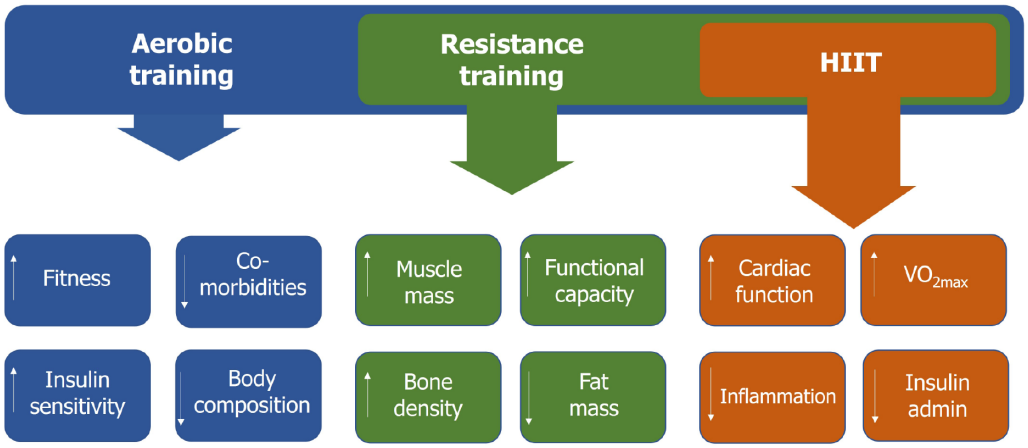

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Figure 1 Main benefits of aerobic training, high-intensity interval training, and resistance exercise in type 1 diabetes mellitus patients.
HIIT: High-intensity interval training.

increase in insulin-independent muscle glucose transport[11]. Thus, insulin secretion in people without a T1DM pathology is reduced. This happens precisely to compensate for the increase in insulin sensitivity and glucose transport caused by physical exercise itself, so the reduction in blood insulin does not restrict the supply of glucose to the muscles[4].

Nevertheless, to maintain metabolic homeostasis and to avoid hypoglycemia, different mechanisms are activated that regulate blood glucose concentration. Four metabolic pathways are triggered to ensure energy production: (1) Glucose mobilization (from glycogen stores) from the liver; (2) fatty acid mobilization from adipose tissue; (3) gluconeogenesis (production of new glucose molecules) from non-carbohydrate (CHO) precursors (amino acids, lactate, and glycerol); and (4) blocking glucose entry into cells and promoting fatty acids (an alternative is oxidation for energy generation) to be used in energy generation[12]. These mechanisms are orchestrated by glucagon, cortisol, growth hormone (GH), epinephrine, and norepinephrine. When the blood glucose concentration decreases, these hormones respond by activating mechanisms to restore the imminent hypoglycemia. Glucagon increases liver glucose production and stimulates gluconeogenesis, while cortisol-GH balance stimulates gluconeogenesis and fatty acid mobilization. Epinephrine and norepinephrine (catecholamines) are responsible for the catabolism of glycogen (glycogenolysis) and lipids (lipolysis) and for reducing muscle glucose consumption. On the other hand, norepinephrine reduces insulin secretion so that it does not interfere with the increase in blood glucose caused by the aforementioned hormones[13].

Important differences in the metabolic behavior of T1DM patients during aerobic exercise must be considered. Furthermore, physical exercise response depends on exercise intensity and volume, CHO intake, as well as type and amount of exogenous insulin[4]. Unlike in the healthy population, during aerobic exercise in T1DM patients exogenous insulin cannot decrease similarly to the pattern of non-T1DM individuals due to non-insulin-dependent muscle glucose transport and insulin sensitivity increase[11]. Moreover, given the pharmacokinetics and peak action of exogenous insulin and considering that exercise intervention is usually performed between 0-4 h after insulin injection, insulin levels are unpredictable. In addition, especially when injected near currently active musculature, insulin can be rapidly absorbed by subcutaneous tissue, rapidly transferring it into the bloodstream when exercise activity is initiated with unforeseeable results[12].

The abnormally high blood insulin levels during physical exercise in T1DM result in an exaggerated entry of glucose into the musculature and the inhibition of endogenous glucose production and fatty acid mobilization mediated by cortisol, GH, glucagon, and catecholamines. Under normal conditions, these hormones act by increasing the blood glucose concentration in the face of low insulin levels, but in T1DM these hormonal mechanisms are impaired[4,12]. Consequently, an excessive drop in blood glucose concentration or even hypoglycemia (< 70 mg/dL) may occur during physical exercise, which depending on its severity can cause dizziness, fainting, and coma. Such hypoglycemic events can still occur hours after the end of physical exercise if appropriate measures are not taken.

After physical exercise, muscle glucose consumption is reduced, but insulin sensitivity remains high. This fact, together with the need to replenish muscle glycogen stores that have been consumed during physical exercise, can lead to post-exercise hypoglycemia and even occur while asleep at night as insulin sensitivity tends to be biphasic (occurring immediately after physical exercise and 7-11 h later). People with T1DM may potentially experience 42-91 hypoglycemic episodes annually. Moreover, approximately 12% of T1DM patients have at least one severe hypoglycemia episode per year[14]. The fear of these episodes makes people with T1DM unwilling to participate in this type of exercise[15].

In summary, the appropriate course of action for people with T1DM in order to be able to safely engage in aerobic physical exercise is based on ensuring an adequate CHO intake prior to physical exercise that elevates blood glucose levels to above 126 mg/dL but not over 270 mg/dL, in tandem with a reduction of insulin dosage before training to counteract the increase in insulin sensitivity and the intensification of non-insulin dependent glucose transport mechanisms occurring during physical exercise[4]. To this end, it is important to take at least two blood glucose measurements, one half an hour before and a second 10 min later. If the physical exercise is long-lasting, an extra supply of glucose and fructose will be essential during the exercise. After the end of physical exercise, insulin reduction and CHO intake is again essential to prevent post-exercise hypoglycemia[16].

When the adjustment in insulin dose and CHO intake becomes imbalanced, diabetic ketoacidosis may occur. In the presence of reduced insulin levels and a high concentration of counter-regulatory hormones such as epinephrine or glucagon, glucose is unable to enter the muscles, among other tissues, and as a result non-esterified fatty acids and glycerol are produced from the catabolism of triglycerides. Glycerol is used as a substrate in gluconeogenesis, but fatty acids catalyzed by carnitine are oxidized to ketone bodies in the liver as an alternative means of obtaining energy. Hyperketonemia may lead to serious health sequalae[17] such as dizziness, vomiting, and nausea, and when severe cerebral edema or myocardial injury may result. It is therefore imperative to adjust insulin dosages suitably to ensure safe exercise activity and avoid complications due to either excess or deficiency of the hormone[18].

High-intensity interval training and T1DM

High-intensity interval training (HIIT) is a type of physical exercise with a recent increase in popularity among fitness enthusiasts (ranked in the top 3 of world fitness trends)[19] and sport science academics alike, with almost 700 publications in PubMed. Despite this recent surge in acclaim, HIIT modalities have been employed in sports performance training since the 1920s[20]. The physiological impact of HIIT has recently been informed in both clinical and sport contexts[21]. HIIT presents a unique opportunity to obtain cardiorespiratory and metabolic benefits comparable to those obtained by classic moderate-intensity continuous training[22] through lower training volumes, addressing the main barrier (lack of time) cited by most people for not doing physical exercise. HIIT consists of performing short-to-moderate (between 8 s and 4 min) bouts of any given physical exercise (mainly endurance exercises) at high intensity (*i.e.*, above the anaerobic threshold) interspersed by brief resting intervals performing low intensity activities such as walking or passive rest periods (ranging from 4 s to 60 s)[23].

Several different HIIT protocols have been proposed throughout the scientific literature based on exercise type, exercise intensity, volume (time duration) and number of exercise intervals, intensity and duration of rest periods, number of sets, length of each set, rest between sets, and exercise intensity during active rest periods[24]. Despite the high variability observed, the considerable majority of HIIT protocols use high-intensity exercise intervals performed between 10 s and 4 min with 30-60-s rest periods between sets. These training programs pursue the accumulation of short bouts of high-intensity exercise (> 90% of VO_{2max}) otherwise not sustainable for long time periods, interspersing short resting periods that allow the high-exertion intervals to be completed at the desired intensity. A complete standard HIIT session usually takes/requires between 20-40 min, including rest periods, of which at least 4 min must be at high intensity (considering the sum of all intervals)[20,25,26].

The anaerobic energy production of HIIT, as high intensity intervals are usually performed above 90% of VO_{2max}, where the initial substrates used are free ATP in the muscle fiber and phosphocreatine determine the acute responses in relation to the metabolism and endocrine system. An aerobic component is also necessary as recovery intervals depend on it[20]. Hence, HIIT has been proposed as a potentially effective tool to improve blood pressure, weight control, glucose regulation, cardiorespiratory fitness, and psychological well-being in chronic pathologies such as hypertension[27], obesity [28], metabolic syndrome[29], T2DM[30], heart failure[31], chronic obstructive pulmonary disease[32], and mental illness[33]. However, despite the benefits HIIT has demonstrated in other chronic diseases. The effect that this type of training has on people with T1DM has not yet been extensively studied[4].

High-intensity stimuli lead to an increase in catecholamine secretion, inhibiting insulin-mediated glucose consumption and accelerating gluconeogenesis. As a result, obtaining energy from glucose without the intervention of oxygen (anaerobic glycolysis), muscle fibers and blood lactate concentrations increase. This process also inhibits insulin-mediated glucose consumption and promotes glucose production by the liver. Taken together, these mechanisms contribute to a much safer glycemic regulation during and after physical exercise in people with T1DM compared with moderate-intensity aerobic exercise, preventing the occurrence of hypoglycemia[1]. In addition, oxygen consumption remains elevated and helps the subject to revert to a regular basal metabolic state after training through lactate clearance, increased cardiopulmonary function, increased body temperature, enhanced catecholamine effect, and glycogen re-synthesis, using lipids as an energy substrate[34].

Despite being an exercise mode that has been little studied in the T1DM population, HIIT seems to have positive cardiovascular and metabolic effects in people with this condition. Reported benefits include increases in VO_{2max}, improvements in vascular function, psychological well-being, body composition, cardiac function, and antioxidant and anti-inflammatory markers, along with a reduction in the amount of insulin administered[35-40] (Figure 1). All the above, along with the prevention of hypoglycemia and the short time required can overcome the major barriers that people with T1DM

present against physical exercise[6,15], positioning HIIT interventions as a useful therapy for this population, it may be a better alternative compared to aerobic or resistance exercise training, which pose a higher risk of hypoglycemia and require more time, although they are not mutually exclusive.

RESISTANCE TRAINING AND T1DM

Resistance exercise guidelines and benefits

Resistance exercise refers to the exercise mode in which muscles produce tension to accelerate, decelerate, or maintain immobility for any given resistance. This resistance could be weights, bands, or even the subject's own bodyweight working against gravity[41]. Depending on training variable manipulation (exercise volume, intensity, mode of contraction, movement velocity, and rest intervals between sets), a specific resistance training program might result in muscle hypertrophy, strength, mechanical power, and endurance enhancements[42]. Resistance training is currently being recommended for patients with T1DM by the American Diabetes Association and the American College of Sports Medicine. The recommendation is performing on 2-3 non-consecutive training days prioritizing large muscle groups, with at least 8-10 exercises in 1-3 sets of 10-15 repetitions at an intensity ranging from 50% to 75% of one-repetition maximum[12,43].

There is a known relationship between skeletal muscle mass and higher-level functional capacity[44]. People with T1DM are susceptible to muscle mass loss and sarcopenia faster than people without this disease, even without having developed disease-specific complications[45]. Resistance training might therefore address those fundamental deficits in this population[46]. Apart from muscle mass increase, one of the main benefits of resistance training in T1DM patients is the improvement of bone density, essential in this population because hyperglycemia in T1DM patients causes bone mineral mass loss earlier than people of the same age, physical condition, and body composition[47,48]. It is also well-known that resistance training improves body composition (*i.e.*, reduced fat mass and increased muscle mass)[49] thus preventing the development of overweightness, lately noted as a prevalent issue in this population[50] (Figure 1). In addition to the significant improvements observed in a functional capacity after accomplishing a resistance training program, another fundamental benefit of resistance training is its impact on cardiovascular health through the improvement in the lipid profile and vascular function [49]. This is relevant for T1DM patients since cardiovascular disease is the leading cause of mortality in this population[51,52]. Moreover, an adequate resistance training program enhances functional capacity by improving daily activity functionality, preventing falls, injuries and cardiovascular diseases, and increasing independence[12,49].

Despite the lack of studies analyzing the acute response to resistance training in people with T1DM [49], it should be noted that the hormonal response and the overwhelmingly anaerobic metabolism cause a much slower reduction in glucose levels during resistance training than that occurring during aerobic exercise in people with T1DM. Similarly, resistance training is associated with a much more stable post-exercise glucose concentration in comparison to aerobic exercise (hypoglycemia during and after exercise), which would be reduced with this exercise mode[53]. The increases in catecholamine concentration during resistance training and consequently the increase in endogenous glucose production allows T1DM patients to more easily adjust exogenous insulin dosage and CHO intake than with aerobic exercise.

However, certain types of resistance training with high volume and low intensity might induce a decreased hormonal response, but resistance training with sufficiently high intensity and low volume is associated with an enhanced hormonal response, leading to higher hepatic glucose production. Moreover, an initial reduction in exogenous insulin or CHO intake before the resistance training program to prevent the drop in blood glucose is not necessary as opposed to what typically occurs with aerobic exercise. Despite this, it may still be necessary to control the hyperglycemic tendency after resistance exercise by increasing the insulin dose and postponing the intake of CHO[4]. However, the acute effect of resistance training in people with T1DM has not been elucidated yet, and more research is warranted to understand the specific underpinning mechanisms of the insulin/CHO ratio in association with different types of resistance training completed[14,49] (Figure 1).

PRACTICAL APPLICATIONS

Conditional and psychological assessment

A comprehensive pre-exercise screening should be performed before designing an individualized training program for each T1DM patient. This should be preferably performed by sports science professionals with proper expertise in T1DM. Prior evaluation should include an anamnesis assessment and physical examination as well as a cardiopulmonary function test. Patients should also be screened for risk factors or presence of cardiovascular, respiratory, or metabolic disorders apart from T1DM. When the medical approval for the implementation of an individualized training program has been obtained,

Table 1 Evaluation protocols in type 1 diabetes mellitus exercise programming

Parameter	Measures	Comments
Aerobic fitness		
Incremental test	Workload and steady-state HR to predict V_{O2peak}; RPE	Treadmill or cyclo-ergometer; Gas collection system and HR monitor necessary. Begin with unloaded warm-up
6-min walking test	Total distance walked, HR, RPE, BP	HR and BP monitor necessary
Muscular strength/power		
Indirect repetitions maximum testing	Maximal weight lifted for < 10 repetitions	Use machines. Remind patients to exhale on concentric action and avoid holding their breath
Force-Velocity profile	Execution velocity at a given load	Encoder necessary
Timed up and go test	Time to stand from a chair, walk a 3-m round trip, and sit back down on the same chair	Results correlate with gait speed, balance, functional level, the ability to go out
30-s sit to stand test	Number of times patient comes to a full stand with arms crossing a standard size chair in 30 s	A functional measure of lower limb strength, power, and muscle endurance
Flexibility/mobility		
Goniometry	Range of motion	Focus on flexibility of hamstrings, hip flexors, ankle plantar flexors, shoulder adductors, and internal rotators
WBLT	Ankle dorsiflexion	No footwear; no equipment
Psychological well-being		
SF-36	Quality of life	Eight-domain profile of functional health and well-being scores
PSQI	Sleep quality	Seven-domain profile of sleep quality and related disorders

BP: Blood pressure; HR: Heart rate; PSQI: Pittsburgh Sleep Quality Index; RPE: Rating of perceived exertion; SF-36: Short Form Health Survey-36; WBLT: Weight-bearing lunge test.

the patient's cardiorespiratory, neuromuscular and functional performance should be tested (Table 1). Similarly, it is important to use tools to assess important psychological aspects such as quality of life and sleep quality, since these are issues that can affect people with T1DM (Table 1).

Practical recommendations for exercise prescription in T1DM patients

An individualized exercise program should be designed to address the patient's goals (*e.g.*, improve strength, endurance, balance, coordination, *etc.*) considering the patient's baseline impairments and capabilities. The exercise program should include all the necessary training variables, such as frequency, volume, intensity, exercise mode, and precautions to be considered, prior to and after the program. It is important to bear in mind that in practice blood glucose levels may show a variable response for the same CHO-insulin adjustments. A multitude of factors, such as the food previously eaten, hours of sleep, and stress, exert varying influences. Consequently, it is necessary that, blood glucose should be analyzed in each training session, and necessary actions should be taken.

At times, it will be necessary to adapt the training to the expected behavior of blood glucose. For example, if a patient with T1DM has forgotten to lower the pre-training insulin dose and aerobic exercise was planned, it will be necessary to modify the training to high-intensity interval work to compensate for the drop in blood glucose that would have occurred with aerobic exercise. On the other hand, if insulin adjustment has not occurred or the patient is at high blood glucose values without circulating insulin, intense resistance training or HIIT should be substituted by aerobic tasks. General recommendations for practical application are shown in Table 2.

A patient's previous experience and training status must be considered when designing any training program. In the first training weeks, the program should focus on basic general conditioning to improve technique in basic resistance exercises, such as squats, lunges, deadlift, and other press and pull movements. The first adaptations to resistance training are acquired with simple exercises (*e.g.*, weight-stack machines or exercises performed with simple materials such as elastic bands). Simultaneously, HIIT performed with low-impact exercises, such as cycling or rowing, is an excellent option since this does not require significant insulin-CHO adjustments and is safe for the lower limb joints. It is essential that the person increases daily activities (*e.g.*, taking the stairs, walking as much as possible, reducing sitting time). Moreover, before each training session, a warm-up consisting of unloaded pedaling or cranking, general joint mobility, and dynamic stretching should be performed. Controlling daily load by quantifying the total training session rating of perceived exertion as well as glycemia levels before each session is recommended.

Table 2 Practical recommendations for exercise prescription in type 1diabetes mellitus patients

Aerobic exercise[1]	HIIT	Resistance exercise
Exercise intensity: Start with an intensity of 40%-70% of VO_{2max} and gradually increase to 60%-80% of maximum heart rate. RPE of 11-13 is recommended	**Exercise intensity:** > 90 VO_{2max}, 90%-95% of maximum heart rate, and an RPE of 15-18	**Exercise intensity:** 50%-75% 1RM, RPE of 7-8. Participants should perform the exercises as fast as possible during the concentric phase (maximal movement intention). A 20% loss in concentric velocity among the repetitions of each set may be established as a limit in the volume at the given intensity
Exercise volume: 10-40 min duration is suggested. At first, it can be divided into three bouts of 10-12 min per session	**Exercise volume:** 12-20 sets. Bouts of 30 s interspersed by 60 s rest (ratio 1:2)	**Exercise volume:** 1-3 sets of 10-15 reps; 8-10 exercises of large muscles are essential
Exercise mode: Low impact cyclo-ergometer, arm ergometer, arm-leg ergometer, aquatic exercise, treadmill walking, rowing, and running	**Exercise mode:** Aerobic exercises such as cycling, running, rowing, *etc*. First, HIIT must be performed in low impact conditions, such as cyclo-ergometer or aquatic environment, aiming for at least a total of 4-min at high intensity	**Exercise mode:** Prioritize lower limb exercises and multi-joint exercises. Exercise velocity must be initially moderated (1-2 s concentric, 1-2 s eccentric)
Training frequency: 1-3 sessions per week; as per patient tolerance	**Training frequency:** 1-3 sessions per week	**Training frequency:** 2-3 sessions per week
Progression: During the first 1-4 mo, progression should be achieved by increasing the duration or frequency of exercise sessions. After this time, test whether higher intensity in continuous exercise is tolerated	**Progression:** Increase total training volume gradually, then increase the density by reducing active rest intervals or increasing the length of the HIIT bouts, as per patient tolerance	**Progression:** Begin with weight-stack machines, elastic bands, and weightbearing exercises. Increase load and progress to more technically demanding exercises. An exercise intensity of resistance can be securely added by 2% to 5% when 15 repetitions can be properly performed in successive training sessions

[1]When the insulin-carbohydrate ratio is cautiously established. 1RM: One-repetition maximum; HIIT: High-intensity interval training; RPE: Rating of perceived exertion.

The ideal scenario would involve the use of continuous glucose monitoring, a relatively new technology that provides real-time knowledge of intra-session and inter-session glucose regulation[54]. Since glucose does not have a mathematical behavior, this technology is of great importance to prevent adverse events during exercise training and in the subsequent hours. In the same way, insulin pumps help to automatically regulate the exogenous administration of this hormone and maintain stable glucose levels, depending on exercise and diet. However, accessibility to continuous glucose monitoring is limited in real scenarios. Hence, it is important to analyze hormonal and metabolic responses to each type of exercise in patients with T1DM to control pre- and post-exercise insulin administration as well as CHO intake.

CONCLUSION

Aerobic and resistance exercise are safe and effective training methods in T1DM patients. Current evidence has shown that a supervised and individualized exercise program with aerobic exercise performed 1-3 times/week, including low-volume high-intensity exercise training along with 1-3 sessions per week of resistance training, is sufficient to improve physical fitness, functional capacity, quality of life, and mental health in this population. These guidelines should be adapted according to the patient's needs, abilities, and preferences.

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FOOTNOTES

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MINIREVIEWS

Diabetes and fatty liver: Involvement of incretin and its benefit for fatty liver management

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Abstract

Fatty liver disease is defined as liver condition characterized by hepatic steatosis, closely related to pathological conditions in type 2 diabetes and obesity. The high prevalence of fatty liver disease in obese patients with type 2 diabetes reached 70%, reflecting the importance of these conditions with fatty liver. Although the exact pathological mechanism of fatty liver disease, specifically non-alcoholic fatty liver disease (NAFLD) remains not completely revealed, insulin resistance is suggested as the major mechanism that bridged the development of NAFLD. Indeed, loss of the incretin effect leads to insulin resistance. Since incretin is closely related to insulin resistance and the resistance of insulin associated with the development of fatty liver disease, this pathway suggested a potential mechanism that explains the association between type 2 diabetes and NAFLD. Furthermore, recent studies indicated that NAFLD is associated with impaired glucagon-like peptide-1, resulting in decreased incretin effect. Nevertheless, improving the incretin effect becomes a reasonable approach to manage fatty liver disease. This review elucidates the involvement of incretin in fatty liver disease and recent studies of incretin as the management for fatty liver disease.

Key Words: Fatty liver; Diabetes; Incretin; Insulin resistance

Core Tip: Type 2 diabetes mellitus (T2DM) is correlated with various metabolic disorders, including fatty liver. The influence of T2DM on incretin hormones contributed to fatty liver development. Impairment in lipid and glucose metabolism, fat oxidation, oxidative stress, and other effects lead to liver fat deposition. Therefore, drugs targeting the incretin hormones may provide beneficial effects on patients.

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INTRODUCTION

Fatty liver disease is a spectrum of inflammatory diseases, ranging from hepatic steatosis to cirrhosis. In a continuous process, it may develop into fibrosis and cirrhosis. The diagnosis of non-alcoholic fatty liver disease (NAFLD) remains challenging since the current definition is a diagnosis of exclusion. Consensus stated that the diagnosis of NAFLD could be made if liver fat accumulates > 5% without any other cause. This makes the diagnosis very challenging due to the influence of other variables. The current definition also suggests that a liver biopsy is required to determine the degree of fat accumulation. The health burden of NAFLD present significant concern, since the prevalence of NAFLD is increased and affects 25% of people globally. The economic impact of NAFLD also become major concern since the financial burden reaching $100 billion *per* year[1].

The pathophysiology of NAFLD is multifactorial, involving metabolic factors. Among all factors contributing to the development of NAFLD, impairment in hormones become important variables to be considered. Impairment of hormones affected lipid and glucose metabolism, interference with other hormones' signaling, and oxidative stress[2]. Among hormones associated with the development of NAFLD, incretin hormones become an interest.

Incretin hormones influence glucose homeostasis and are involved with the pathophysiology of type 2 diabetes mellitus (T2DM). Incretin hormone is a gut peptide which secreted after nutritional intake. Incretin hormones consist of GIP (glucose-dependent insulinotropic polypeptide) dan GLP-1 (glucagon-like peptide-1). Both affect lipid metabolism, insulin release, oxidative stress, and other factors associated with glucose metabolism. This important aspect of incretin hormones makes it involved in other metabolic diseases, including NAFLD. Hence, it also served as the target to improve the outcome of metabolic diseases. In this review, we elaborate on the mechanism of incretin hormones and the reported recent studies which evaluate the clinical aspect of incretin hormones in NAFLD[3,4].

THE WORK OF INCRETIN HORMONES

The work of incretin hormones, known as the incretin effect, works more effectively when the glucose is administered orally compared to administered intravenously (two to three times more effective). Other substances are also involved in the mechanism of incretin hormone; inhibitors of dipeptidyl peptidase-4 (DPP-4 inhibitors) involved in the therapeutic efficacy of incretin effects. The DPP-4 inhibitors increase the concentration of GLP-1[3,4].

Oral glucose intake leads to an increment of insulin secretion stimulation compared to intravenous glucose infusion. This effect occurred even though the iso glycemic condition was reached[5]. This phenomenon occurred because of incretin hormone release (GIP and GLP-1) after oral glucose intake from the gut entero-endocrine. This condition did not occur after intravenous glucose infusion[5,6]. The secreted incretin hormones acted as endocrine signals to the pancreatic islet of Langerhans. These lead to the increment of insulin secretion and glucagon secretion modulation when glucose concentration is above 66 mg/dL.

Pancreatic β-cells have GIP and GLP-1 receptors in their membrane. In the event of the binding of its receptor with its ligands, the activated receptors will bind with adenylate cyclase. This resulted in increased cyclic adenosine monophosphate (AMP) production, leading to protein kinase A activation[7,8]. However, this signaling pathway did not release pre-formed insulin secretory granules from pancreatic β-cells. In order to release the granules, the closure of the potassium channel, depolarization, and calcium ion influx initiated by the hyperglycemic condition is needed. Therefore, the effects of an increase in insulin release due to incretins always require hyperglycemia in certain limits (66 mg/dL)[9].

Another effect of incretin hormone is glucagon release. GIP molecule stimulates glucagon release, particularly in decreased glucose concentration, while GLP-1 suppresses glucagon secretion in hyperglycemia, resulting in hepatic glucose production[10,11]. The mechanism of incretin hormones in

the liver is indirectly mediated since no GLP-1 receptors exist. The mechanism responsible for this phenomenon is the autonomous nervous system.

The incretin hormones possess additional biological effects on other organs. GLP-1 hinders appetite and food intake. GLP-1 also increases satiety. The GLP-1 receptors were observed in the hypothalamus [12]. GLP-1, derived from blood flow circulation, enter the brain through the circumventricular organ, characterized by a leaky blood-brain barrier. Therefore, GLP-1, with chronic stimulation of its receptor, is considered a signal to suppress appetite, which acts as a basic mechanism for a decrease in body weight[4,13].

Another additional effect of incretin hormone is the triglycerides storage in adipose tissue. GIP induces lipoprotein lipase, an enzyme that releases fatty acid from triglycerides chylomicrons in adipose tissue; hence it eliminates triglycerides chylomicrons. However, this is still based on animal studies; it is still uncertain whether the same occurred in humans[14,15].

Gastric emptying is also affected by GLP-1 but not by GIP[16,17]. The consequence of this effect is the nutritional delivery to the intestinal lumen is hampered. The decreased absorption of nutrition resulted in the stagnant increase of blood glucose and triglycerides after a meal[18].

Other effects of incretin include bone metabolism and cardiovascular function. Regarding bone metabolism, animal study of GIP found that the signaling pathway through GIP receptors inhibits bone resorption, both from the amount and the function of osteoclast, and supports bone formation (osteoblast function)[19]. The effect of incretins on the cardiovascular system is related to their role in cardiac blood supply, vasodilatation, inflammation response in adipose tissue and blood vessels, substrate intake, cytokine release and atherosclerosis formation, and plaque stabilization[20,21].

It should be noted that the dogma of proglucagon produced in α-cells of the pancreas and GLP produced by intestinal L cells has been challenged. It has been suggested that after total pancreatectomy, glucagon produced by intestinal cells and GLP-1 exist in pancreatic α-cells[22,23]. The animal study suggested that GLP-1 produced by pancreatic α-cells have more potent effect on glucose homeostasis than intestinal cells-produced GLP-1[24]. This showed that the physiological mechanism of the incretin hormones is not as simple as it is known currently. The mechanism of glucagon formation by pancreatic α-cells primarily mediated by prohormone convertase (PC) 2[25], while PC 1/3 acts as the main prohormone for the formation of GLP-1 and GIP[26,27]. It has been suggested that irregular expression of PC 1/3 in the pancreas and PC2 in the intestinal becomes a reason for the existence of GLP-1 in the pancreas[23,28,29] and glucagon in intestinal (Figure 1)[22,30].

The intracellular mechanism of incretin hormone started with the binding of GIP and GLP-1 with their respective receptors, GIP receptors and GLP-1 receptors. It resulted in the activation of adenylate cyclase and the increase of intracellular cyclic adenosine monophosphate (cAMP), leading to protein kinase A (PKA) activation and protein activated by cAMP2 (EPAC2). The activation of PKA induces the closure of the adenosine triphosphate-sensitive potassium channel and facilitates membrane depolarization and the prolongation of potential action. Depolarization opens the voltage-gated Ca^{2+} channel, which leads to an increase in intracellular Ca^{2+}. The increased Ca^{2+} concentration triggers the fusion of insulin-containing granules with the plasma membrane and insulin secretion from pancreatic β cells. The increase of Ca^{2+} levels also drives the transcription of the proinsulin gene, therefore increasing the insulin content of β cells. Furthermore, the activation of EPAC2 increases the density of insulin-containing granules near the plasma membrane to potentiate the secretion of insulin from β cells (Figure 2)[31].

DEVELOPMENT OF FATTY LIVER DISEASE

The pathophysiology of fatty liver disease related to metabolic factors, including NAFLD, is intricate due to its multifactorial nature and related to various comorbidities. The accumulation of liver fat is caused by the imbalance in fatty acid influx (lipolysis of fat tissue), fat disposition (fatty acid disposition), lipogenesis hepatic de novo and very low density lipoprotein secretion by the liver[32]. The progressivity of fatty liver disease involves the interaction of cellular stress response (lipotoxicity and increase of oxidative stress)[33] and liver fat accumulation along with cytotoxicity[33]. The association of gut and hormones released from the pancreas, insulin resistance in muscle, adipose tissue and liver, and gut microbiome are also involved in the pathophysiology of NAFLD. Obesity contributes to fatty liver disease by causing adipocyte hypertrophy and hypoxia, resulting in macrophage influx and pro-inflammatory conditions[34]. The pro-inflammatory condition causing the development of insulin resistance leads to hepatic steatosis. The insulin resistance increases lipolysis and causes the increase of free fatty acids. Hepatic lipotoxicity is caused by the increment of long-chain fatty acids, diacylglycerol, and ceramide, which stored in the liver, causing the release of reactive oxygen species. These contributed to inflammation and liver fibrosis, along with the apoptosis of hepatocytes. Moreover, the increase in hepatic steatosis leads to the resistance of the liver toward insulin, worsening the condition [35].

Type 2 diabetes and metabolic syndromes are closely related to NAFLD[36]. Individuals with T2DM possess a five times greater risk of NAFLD and a greater likelihood to progress toward non-alcoholic steatohepatitis (NASH) when compared to people without T2DM[37]. However, liver steatosis is partly

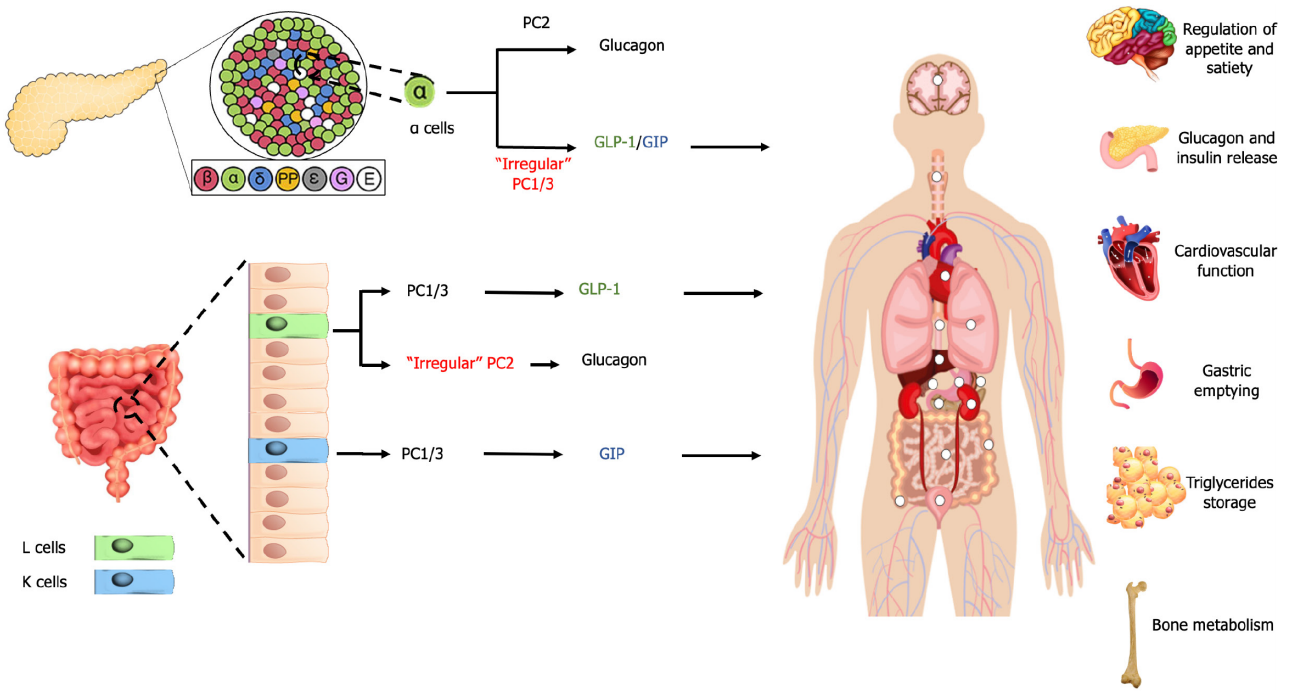

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Figure 1 Production of incretin and its benefits. Glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide produced in the pancreas and intestine mediated by prohormone convertase. The incretin hormones affect appetite and satiety, glucagon and insulin release, cardiovascular function, gastric emptying, triglycerides, and bone metabolism. GIP: Glucose-dependent insulinotropic polypeptide; GLP-1: Glucagon-like peptide-1; PC1/3: Prohormone convertase 1/3; PC2: Prohormone convertase 2.

an adaptive and protective response; lipotoxic free fatty acids is stored as a more stable component. However, this protective nature becomes weakened with continuous liver problems along with other contributed factors, *e.g.*, T2DM and genetic predisposition. This, in turn, causes hepatocyte injury and fibrosis[38]. Insulin resistance in the liver is caused by proinflammatory cytokine (tumor necrosis factor α, interleukin-6), proinflammatory pathway, *e.g.* c-Jun and nuclear factor-kappaB, endoplasmic reticulum stress, and lipid metabolism product.

DIABETES, INCRETIN HORMONE AND FATTY LIVER DISEASE

Incretin hormones are secreted in T2DM patients as well as healthy individuals and obese patients. An early study showed a slight increase of GIP in patients with T2DM and decreased response to GLP-1[39,40], while subjects with impaired glucose tolerance have an intermediate response to GLP-1. Therefore, it has been hypnotized that there is a progressive loss in GLP-1 secretion along with the severity of T2DM. Study has been conducted to compare the secretion of GIP and GLP-1 between healthy and T2DM subjects after oral glucose loads administration and mixed food. There is a slight difference in which lower secretion in T2DM patients. However, another study also found no difference in GIP and GLP-1 between those two populations. A meta-analysis study showed no difference in the secretion of GIP and GLP-1 after nutrition loads between T2DM and healthy subjects[41-43].

Even though the excretion of incretin is approximately normal in T2DM patients, the difference in the characteristic between T2DM and healthy subjects exist in the insulinotropic activity of GIP and GLP-1. GIP is considered a drug candidate for the development of a glucose-lowering agent. In this regard, there is no doubt that physiological and pharmacological concentrations of GLP-1 also exhibit insulinotropic features in T2DM patients[10]. Inappropriate response to GIP may explain the lower effects of incretin hormones in T2DM patients compared to healthy subjects[10,44]. Previously conducted studies have found that the reduced incretin effects occurred after the diagnosis of T2DM was confirmed. Hence it has been suggested that the decrease in incretin effects is secondary to this condition[45]. It is still not fully elucidated which features of T2DM, *e.g.*, inflammatory infiltration of β-cells, hyperglycemia, islet lipid overload, or other mechanisms may trigger this phenomenon[5,45]. The reduced expression of GIP receptors or substances involved in the GIP signaling pathway is also suggested to explain the impairment in insulin secretion[46]. Although animal study with diabetic hyperglycemia has found that GIP receptors are decreased, the same is not found in the human pancreas. In conclusion, type 2 diabetes condition reduces the incretin effect and worsens glycemic control. This situation leads to glucotoxicity. Glucotoxicity resulted in a reduction of beta cell mass in the pancreas and reduced

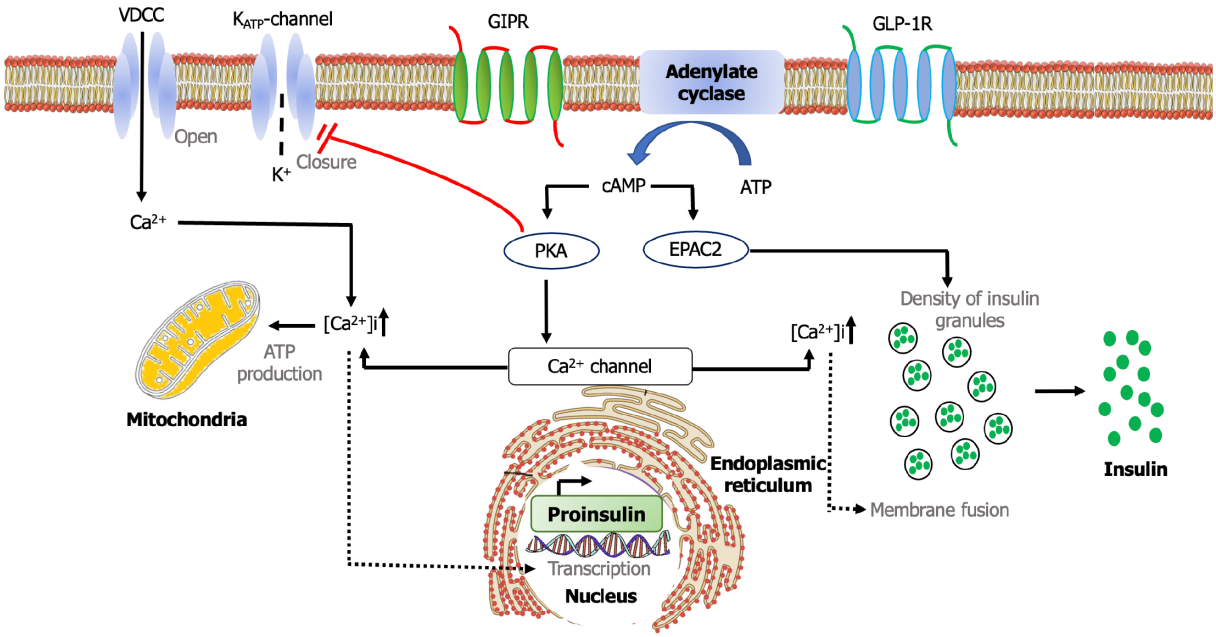

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Figure 2 Intracellular mechanism of incretin hormones. The binding of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 with their respective receptors increase cyclic adenosine monophosphate and activation of protein kinase A. This result in the increase of Ca^{2+} levels, mediating the fusion of insulin-containing granules with the plasma membrane and insulin secretion from pancreatic β cells. ATP: Adenosine triphosphate; cAMP: Cyclic adenosine monophosphate; Ca^{2+}: Calcium; [Ca2+]i: Calcium influx; EPAC2: Exchange protein activated by cAMP2; GIPR: Glucose-dependent insulinotropic polypeptide receptor; GLP-1R: Glucagon-like peptide-1 receptor; K_{ATP}-channel: ATP-sensitive potassium channel; K^+: Kalium; PKA: Protein kinase A; VDCC: Voltage-gated calcium channels.

expression of GIP receptors. These will further reduce the incretin effect, creating a vicious cycle. Numerous studies with insulin treatment to control hyperglycemia to reach a near-normal value of glucose concentrations have been done. Insulin treatment may improve the insulinotropic of GIP and GLP-1 in T2DM patients, therefore leads to improvement of the incretin effects[47,48].

The reduced incretin effects may result in further damage of hepatocytes. Reduced incretin effects may reduce satiety and caloric intake, resulting in increased body weight. The increase in body weight leads to adipose tissue insulin resistance, increased lipolysis and leptin, and decreased adiponectin. The final result leads to increased hepatic insulin resistance, de novo lipogenesis, and hepatic fat deposition. Reduced incretin effects also lead to reduced insulin release, resulting in increased adipose tissue insulin resistance and increased hepatic insulin resistance and hepatic fat deposition. Another mechanism of decreased incretin is increased dietary fats and chylomicrons, resulting in increased hepatic fat deposition (Figure 3)[2].

An approach to modulate the expression and activity of incretin hormones may benefit fatty liver disease. The effect of incretin could improve the satiety, therefore reduced caloric intake. The insulin resistance could be improved, leading to downregulation of lipid in liver, lipotoxicity and oxidative stress, providing beneficial effect in NAFLD patients.

CLINICAL ASPECT OF INCRETIN IN FATTY LIVER DISEASE

The primary treatment of fatty liver, particularly NAFLD, is decreasing body weight. The decrease of body weight by 10% with regulating diet and physical activity decreases the triglycerides concentration by 60% in overweight people[49]. Another modality is bariatric surgery for patients with severe obesity. This modality may significantly improve lobular inflammation and NASH in 50%-85% of cases[50]. Management with pharmacologic agents remains explored to discover the agent that can give significant efficacy. In short, the pharmacological agents may be classified into agents to improve metabolic impairment, including body weight, inflammation with oxidative stress and dysregulation in the gut-liver axis[51]. In regards to those specific points, the pharmacological agent is ideally able to work in all those mechanisms.

A study showed that GLP-1 had the effect of inducing satiety through the central mechanism in hypothalamus and brain stem. The use of GLP-1 also decreases caloric uptake. These results were obtained from observation of person with obesity and T2DM. A decrease in body weight is also a consistent discovery obtained from clinical trials with GLP-1 receptor agonists (GLP-1RAs). Chronic use of GLP-1 is also expected to improve insulin sensitivity since it is related to its effect on decreasing body

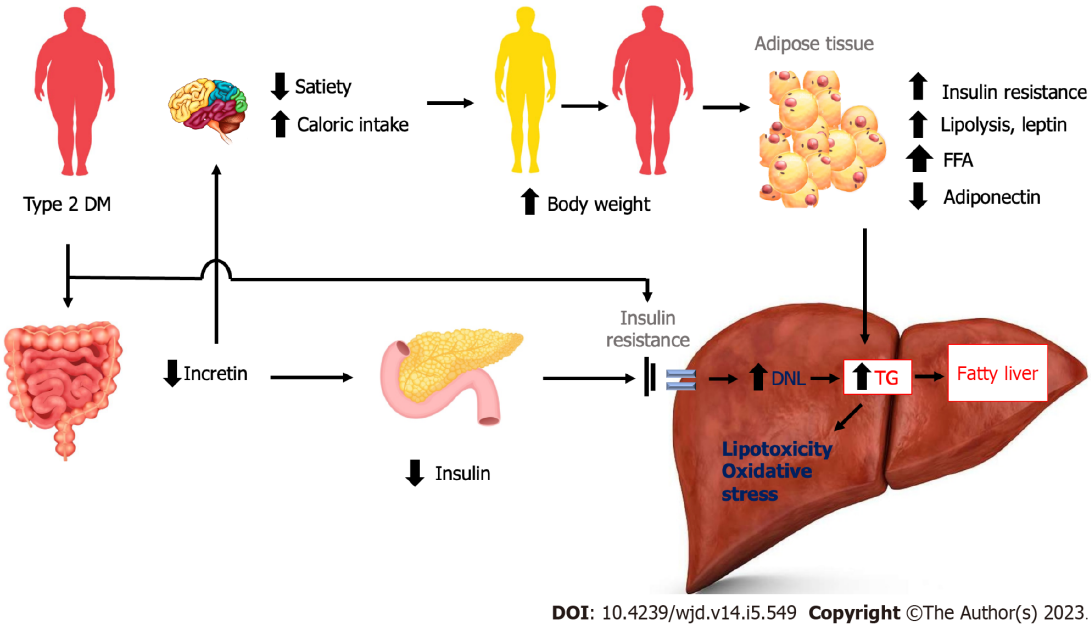

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Figure 3 The effect of type 2 diabetes mellitus on incretin hormone and the development of non-alcoholic fatty liver disease. Increased body weight as the result of reduced incretin effects leads to adipose tissue insulin resistance, increased lipolysis and leptin, and decreased adiponectin, resulting in hepatic fat deposition. DM: Diabetes Mellitus; DNL: De novo lipogenesis; FFA: Free fatty acids; TG: Triglycerides.

weight. Other effects of GLP-1RA administration in NAFLD patients are also related to increased total adiponectin serum concentration and improvement of dysfunctional adipose tissue[52]. Liraglutide also decreases fasting leptin serum levels. Adiponectin is able to repair liver impairment related to fatty liver injury by regulating liver fatty acid oxidation and activity of acetyl-CoA carboxylase as well as fatty acid synthase, which acts as the main enzyme to synthesize fatty acid[53]. The randomized controlled trials of several studies already conducted on the effects of GLP-1RAs toward fatty liver conditions are summarized in Table 1.

Dual incretin receptor agonists are new pharmacological agents that act on GLP-1 and GIP receptors [54]. The new dual incretin receptor agonists have a synergistic effect. The synergistic effects of these pharmacologic agents lead to reduced total liver fat content, risk of cardiovascular disease, body weight and blood glucose levels [determined by glycated hemoglobin, or hemoglobin A1c (HbA1c)][55].

The clinical aspect of dual incretin receptor agonists has been showed in several studies. Tirzepatide, the dual receptor agonist which administered subcutaneously, was approved by the United States US Food and Drug Administration for glycemic control in T2DM patients, In May 2022[56]. Tirzepatide, compared to semaglutide and insulin, showed a greater reduction of HbA1c[56]. A study by Hartman *et al*[57] in 2020 showed that tirzepatide reduce several biomarkers of steatohepatitis, including N-terminal type III collagen propeptide, keratin-18, aspartate aminotransferase, and alanine aminotransferase. The study also showed the increase of adiponectin levels. A phase 2b, 26-wk trial of tirzepatide in T2DM patients showed superior effect of tirzepatide compared to dulaglutide in terms of glucose control and reduction in body weight[58]. Tirzepatide of 5 mg, 10 mg, and 15 mg decrease HbA1c levels by 1.6%, 2.0%, and 2.4%, respectively. When compared to 1.5 mg of dulaglutide administration, the decrease of HbA1c only 1.1%. A total of 48% of patients achieved normoglycemia (HbA1c 5.7%) compared with 2% of subjects treated with dulaglutide[58].

CONCLUSION

In conclusion, incretin hormones affect various signaling and mechanisms of lipid and glucose metabolism, insulin release, regulation of glucagon, oxidative stress, the central mechanism of satiety, and various other effects, involved in the development of NAFLD. The importance of the incretin effect on the development and progressivity of NAFLD makes it an ideal target for its management. Clinical research has provide evidence toward beneficial effect on liver content and other metabolic parameters. Further recommendations for drugs targeting the regulation of the incretin effect need to be considered in future studies. Also, future studies on the adverse events of incretin modulation for fatty liver disease should be directed, therefore its safety could be emphasized.

Table 1 Randomized-controlled trials of glucagon-like peptide-1 receptor agonists related to fatty liver diseases

Ref.	Subjects	Intervention (dose)	Comparator	Effects
Jendle *et al* [59], 2009	T2DM	Liraglutide (1.8-1.2-0.6 mg/day) with additional metformin administration	Glimepiride 4 mg or placebo with metformin	10% attenuation ratio of liver-spleen
Fan *et al*[60], 2013	Overweight T2DM	Exenatide (2 x 10μg)	Metformin	Decrease in liver enzyme
Shao *et al*[61], 2014	Overweight/obese T2DM	Exenatide (2 x 10 μg)	Insulin glargine	Decrease of liver enzymes and degree of fatty liver on ultrasound
Tang *et al*[62], 2015	Overweight/obese T2DM	Liraglutide 0.6 to 1.8 mg/day	Insulin glargine	No difference in the decrease of liver fat
Armstrong *et al*[63], 2016	Overweight/obese (17 out of 52 subjects with T2DM)	Liraglutide (1.8 mg/day)	Placebo	Improvement in NASH histology by 39%
Smits *et al* [64], 2016	Overweight/obese T2DM	Liraglutide (1.8 mg/day)	Sitagliptin, placebo	No difference in liver fat content
Dutour *et al* [65], 2016	T2DM	Exenatide 5-10 mcg twice a day	Placebo	Significant decrease in body weight and liver fat content in the exenatide group
Khoo *et al* [66], 2017	Obesity patients without T2DM	Liraglutide (3 mg/day)	Lifestyle intervention	No difference in reducing liver fat
Feng *et al*[67], 2017	T2DM	Liraglutide (1.8 mg/day)	Metformin or glicazide	Improvement in hepatic/renal index ratio
Frøssing *et al* [68], 2018	Women with PCOS and NAFLD	Liraglutide 1.8 mg/day	Placebo	Decrease of body weight by 5.2 kg (5.6% from baseline), liver fat content by 44%, decrease the prevalence of NAFLD by about two-thirds and decrease of fasting blood glucose
Yan *et al*[69], 2019	T2DM and NAFLD	Liraglutide 1.8 mg/day	Insulin glargine and sitagliptin	Decreased liver fat content, reduction of HbA1c levels in all groups, decrease in body weight
Khoo *et al* [70], 2019	Obese and NAFLD	Liraglutide 3.0 mg/day	Lifestyle changing	The two groups had decrease of liver fat content
Liu *et al*[71], 2020	T2DM and NAFLD	Exenatide 1.8 mg/day	Insulin glargine	Decrease of liver fat content, greater reduction of visceral adipose tissue
Bizino *et al* [72], 2020	T2DM and NAFLD	Liraglutide 1.8 mg/day	Placebo	Reduced body weight, but the liver content was not different
Kuchay *et al* [73], 2020	T2DM and NAFLD	Dulaglutide 1.5 mg/week	Placebo	Control-corrected absolute change in liver fat content of -3.5% and relative change of -26.4%
Newsome *et al*[74], 2020	NASH and liver fibrosis	Semaglutide 0.1 mg/day, 0.2 mg/day, and 0.4 mg/day	Placebo	A higher percentage of NASH resolution without worsening of fibrosis, dose-dependent decrease of serum ALT and AST, and higher mean percentage weight loss

ALT: Alanine transaminase; AST: Aspartate transaminase; HbA1c: Hemoglobin A1c; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; PCOS: Polycystic ovary syndrome; T2DM: Type 2 diabetes mellitus.

FOOTNOTES

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COVID-19 vaccination and diabetic ketoacidosis

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Abstract

An efficient coronavirus disease 2019 (COVID-19) vaccine is urgently required to fight the pandemic due to its high transmission rate and quick dissemination. There have been numerous reports on the side effects of the COVID-19 immunization, with a focus on its negative effects. Clinical endocrinology is extremely interested in the endocrine issue that arises after receiving the COVID-19 vaccine. As was already mentioned, after receiving the COVID-19 vaccine, many clinical problems could occur. Additionally, there are some compelling reports on diabetes. After receiving the COVID-19 vaccine, a patient experienced hyperosmolar hyperglycemia state, a case of newly-onset type 2 diabetes. There has also been information on a potential connection between the COVID-19 vaccine and diabetic ketoacidosis. Common symptoms include thirst, polydipsia, polyuria, palpitations, a lack of appetite, and weariness. In extremely rare clinical circumstances, a COVID-19 vaccine recipient may develop diabetes complications such as hyperglycemia and ketoacidosis. In these circumstances, routine clinical care has a successful track record. It is advised to give vaccine recipients who are vulnerable to problems, such as those with type 1 diabetes as an underlying illness, extra attention.

Key Words: Diabetes; COVID-19; Vaccine; Ketoacidosis; Effect

Core Tip: There has also been information on a potential connection between the coronavirus disease 2019 (COVID-19) vaccine and diabetic ketoacidosis. Common symptoms include thirst, polydipsia, polyuria, palpitations, a lack of appetite, and weariness. In extremely rare clinical circumstances, a COVID-19 vaccine recipient may develop diabetes complications such as hyperglycemia and ketoacidosis.

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INTRODUCTION

Because of the pandemic's high transmission rate, an effective coronavirus disease 2019 (COVID-19) vaccine is urgently needed[1]. The available literature indicates that both vaccines help prevent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. However, given that the vaccination is new, any potential side effects are of greater concern[2-3]. When a handful of novel vaccines created in response to the COVID-19 pandemic got emergency approval and were widely distributed in late 2020[2], pharmacovigilance was unwittingly thrust into the spotlight. An effective global post marketing safety surveillance system was emphasized due to the employment of cutting-edge technologies and the anticipated rapid and widespread deployment of the vaccinations. The vaccinations went through extensive clinical evaluation and regulatory authority review. Many reports on the adverse effects of the COVID-19 vaccination have focused on how diverse they are. Clinical endocrinology is quite concerned about the endocrine issue that manifests after receiving the COVID-19 vaccination. The main concern expressed by the authors of this paper is that diabetes can become a medical problem after receiving the COVID-19 vaccine. After getting the COVID-19 vaccination, numerous clinical issues could arise, as was already mentioned. There are also some interesting reports regarding diabetes. The key words are provided here with a brief explanation.

Diabetes and COVID-19 have a well-established association. There is a bidirectional causal relationship between COVID-19 and type 2 diabetes. Diabetes may exacerbate COVID-19 severity, and COVID-19 vulnerability may increase diabetes risk[4]. Diabetes patients should receive the COVID-19 vaccine, just like everyone else, to protect themselves from the disease. It is critical to discuss the risks of vaccination for those who currently have diabetes mellitus. Piccini *et al*[5] evaluate the likelihood of glycemic control modification, insulin dose adjustment, and adverse effects following COVID-19 vaccination in young people with type 1 diabetes who use varying degrees of technology[5]. Piccini *et al*[5] came to the conclusion that receiving the OVID-19 immunization did not significantly increase the risk of glycemic control disturbance in type 1 diabetes adolescents and young adults[5]. This information may be helpful clinically[6] when counseling families about the SARS-CoV-2 vaccine for young people with type 1 diabetes. In a study by D'Addio *et al*[6] that investigated the immunogenicity and security of SARS-CoV-2 mRNA vaccines, a cohort of individuals with type 1 diabetes took part[5]. The vaccination demonstrated both dependability and security, according to D'Addio *et al*[6].

Several reports claim that COVID-19 vaccine recipients have problems with their diabetes. The exacerbation of hyperglycemia in people with type 2 diabetes after receiving the COVID-19 vaccination is the first problem that needs to be addressed[7]. Mishra *et al*[7] claim that an early inflammatory reaction to the vaccine and a subsequent immunological response are likely to be the causes of a minor and transient rise in blood sugar levels[7]. Mishra *et al*[7] published a case series that substantiated the etiology of transient immuno-inflammation because all episodes of hyperglycemia were self-limited and did not require significant treatment modifications[7]. A rapid jump in blood sugar levels appears to be caused by a vaccine. The possibility of a mild to moderate rise in blood sugar levels following vaccination has been theorized[7]. One patient experienced new-onset type 2 diabetes after receiving the COVID-19 vaccine, which is known as hyperosmolar hyperglycemia state[8].

COVID-19 VACCINATION AND DIABETIC KETOACIDOSIS

Clinical diabetology has an intriguing discussion regarding the COVID-19 vaccine and diabetic ketoacidosis. As was already indicated, the immunization may cause hyperviscosity and have unintended side effects. Additionally, reports of a connection between the COVID-19 immunization and diabetic ketoacidosis have been made. Three days after the first dose of COVID-19 RNA-based vaccines, the patient typically experiences thirst, polydipsia, polyuria, palpitations, a lack of appetite, and exhaustion without a prior history of diabetes[9]. Hyperglycemia, anion gap metabolic acidosis, and ketonuria are the three main signs of classic diabetic ketoacidosis[9]. It is possible to detect insulin autoantibody positivity and latent thyroid autoimmunity[10]. Ganakumar *et al*[11] advised that people with diabetes, particularly those with type 1 diabetes mellitus and inadequate glycemic control, be constantly monitored for hyperglycemia and ketonemia for at least two weeks after receiving the COVID-19 vaccine[11]. Autoimmunity and genetic predisposition may have contributed to the onset of the disease, even if the precise pathophysiologic mechanisms underlying type 1 diabetes are still unknown[12].

Table 1 Table summarizing the key information of coronavirus disease-19 vaccine related diabetic ketoacidosis in recipients with background type 1 and type 2 diabetes mellitus

Characteristics	Cases with background type 1 diabetes mellitus	Cases with background type 2 diabetes mellitus
Sex	Usually male	Usually male
Age group	Adolescent	Elderly
Background diabetes control	Poor control	No significant relationship
During of diabetic illness	Long	No significant relationship

According to Tang *et al*[12], vaccination could result in type 1 diabetes, irreversible islet beta cell loss, and autoimmunity in persons with susceptible genetic backgrounds[12]. The problem might be more serious and more likely to occur in situations where type 1 diabetes is already present. Yakou *et al*[13] advised that the immunization be cautiously administered to type 1 diabetes patients receiving strict insulin therapy and a sodium-glucose transporter[13] due to the increased risk of ketoacidosis. In the affected case, despite hyperglycemia and diabetic ketoacidosis (DKA) after SARS-CoV-2 immunization, low glycohemoglobin levels are a crucial indicator of COVID-19 vaccine-related DKA[14]. As a preventive measure, it is essential to counsel patients to continue getting insulin injections[13]. Due to the significant risk of ketoacidosis, the vaccination should be cautiously given to type 1 diabetes patients receiving rigorous insulin therapy and a sodium-glucose transporter[15]. When a patient becomes ill, it's crucial to remind them to continue taking their insulin injections and to drink enough fluids[13]. A similar preventative concern should be used in the case of the patient with poorly controlled type 2 diabetes, in addition to the patient with underlying type 1 diabetes. According to Kshetree *et al*[15], Type I or dysglycemia in Type 2 diabetes mellitus is becoming more frequently documented following COVID-19 vaccinations or infection[16]. The mechanisms could be autoimmunity following mRNA vaccinations, cytokine-mediated beta-cell injury, or as a component of an autoimmune syndrome brought on by vaccine adjuvants[15]. Further investigation into the negative effects of people prone to life-threatening illnesses is required, as suggested by Lin *et al*[14]. Also, there might be a need for postvaccination surveillance on both hyperglycemia and DKA problems[16].

Concerning the reported cases of a link between COVID-19 vaccination and diabetes ketoacidosis, an important clinical question is whether ketosis in type 1 diabetes is related to the use of sodium-glucose transport protein 2 (SGLT2) inhibitors. The clinical history of the vaccine recipients in the published articles on the clinical association usually revealed no use of SGLT2 inhibitors, which could be a clue to support the possible clinical association between COVID-19 vaccination and ketoacidosis. Last but not least, it should be noted that the mRNA COVID-19 vaccine is primarily associated with most findings on the relationship between COVID-19 immunization and diabetic ketoacidosis. There are, however, a few reports of clinical associations with other vaccination types (viral vector and inactivated COVID-19 vaccines) that have been documented[11]. The fact that the mRNA vaccination is currently the primary recommended COVID-19 vaccine may be the cause of the higher number of reported cases in the mRNA vaccine group. As previously stated, the COVID-19 vaccination may cause diabetic ketoacidosis in patients with type 1 or type 2 diabetes mellitus (Table 1).

There are significant differences in COVD-19 vaccine-induced diabetes ketoacidosis between recipients with type 1 and type 2 diabetes. COVID-19 vaccine induced diabetes ketoacidosis usually occurs in adolescent male cases with inadequate glycemic control in cases with background type 1 diabetes mellitus[11]. This is the same pattern seen in diabetic ketoacidosis caused by COVID-19 in type 1 diabetes patients[17]. There are fewer reported cases of COVID-19 vaccine-induced diabetes ketoacidosis in people with type 2 diabetes mellitus, and the patient is usually an elderly man with a long history of diabetic illness[15]. The background hemoglobin A1C level, on the other hand, has not been identified as a risk factor for the development of COVID-19 vaccine-induced diabetic ketoacidosis [18].

CONCLUSION

In general, the COVID-19 immunization should be given to the diabetic patient because it has been proven to be effective. Generally, it has been confirmed that it is secure. In exceedingly uncommon clinical situations, a COVID-19 vaccination recipient may experience diabetes-related problems such as hyperglycemia and ketoacidosis. Routine clinical care has a history of success in some situations. Users of vaccines who are more likely to develop problems, such as those who already have type 1 diabetes as an underlying illness, are advised to receive additional attention. Because there is a possible link between the COVID vaccine and ketoacidosis, the risk diabetic case must be closely monitored. There is still a need for more clinical research on this subject because there isn't any *in vivo* or *in vitro* experimental data at this time.

FOOTNOTES

Author contributions: Joob B and Wiwanitkit V contributed equally to this work; Joob B and Wiwanitkit V give the ideas; Joob B wrote and analyzed the data; Wiwanitkit V supervised; All authors have read and approve the final manuscript.

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Exercise therapy for sarcopenia and diabetes

Seung-Taek Lim, Sunghwun Kang

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Abstract

Aging is characterized by the gradual deterioration of function at the molecular, cellular, tissue, and organism levels in humans. The typical diseases caused by changes in body composition, as well as functional decline in the human body's organs due to aging include sarcopenia and metabolic disorders. The accumulation of dysfunctional aging β cells with age can cause decreased glucose tolerance and diabetes. Muscle decline has a multifactorial origin, involving lifestyle habits, disease triggers, and age-dependent biological changes. The reduced function of β cells in elderly people lowers insulin sensitivity, which affects protein synthesis and interferes with muscle synthesis. The functional decrease and aggravation of disease in elderly people with less regular exercise or physical activity causes imbalances in food intake and a continuous, vicious cycle. In contrast, resistance exercise increases the function of β cells and protein synthesis in elderly people. In this review, we discuss regular physical activities or exercises to prevent and improve health, which is sarcopenia as decreased muscle mass and metabolic disorders as diabetes in the elderly.

Key Words: Elderly; Diabetes; Sarcopenia; Resistance exercise; Aging; Muscle

Core Tip: Exercise or physical activity should be regularly performed even before aging begins, and muscle mass should be increased through resistance exercise. The protein intake necessary for protein synthesis during resistance exercise should also be maintained in elderly people and those with diabetes or/and sarcopenia.

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INTRODUCTION

Aging is characterized by the gradual deterioration of function at the molecular, cellular, tissue, and organism levels, and human age is a major risk factor for diseases, including cardiovascular disease, diabetes, osteoporosis, and cancer[1]. Also, gradual decreases in muscle mass, especially in the lower extremities, and increases in fat volume, especially visceral and intermuscular fat, are general body composition changes associated with aging[2]. The typical diseases caused by changes in body composition (decreased muscle mass and increased fat mass), as well as functional decline in the human body's organs due to aging, include sarcopenia and metabolic disorders. Moreover, according to a recent estimate by the International Diabetes Federation, 8.8% (425 million people) of the world's 20-79-year-old population suffered from diabetes in 2017, and the number is expected to rise to 9.9% (629 million people) in 2045[3].

Elderly has complex diseases, not single diseases. Most review studies focus only on a single disease. In addition, it has been suggested that sarcopenia in the elderly plays a pivotal role in the pathogenesis of the frailty and functional disorders in diabetes. Through this review, we discuss regular physical activities or resistance exercises to prevent and improve health, which are sarcopenia as decrease muscle mass and metabolic disorders as diabetes in the elderly.

CAUSES OF DIABETES DUE TO AGING

Several factors are involved in the high prevalence of type 2 diabetes (T2D) in elderly people: (1) In relation to aging, T2D is associated with the decreased function of β cells that secrete insulin and decreased insulin sensitivity[4]; and (2) changes in the body composition related to aging lead to changes in insulin sensitivity due to a decrease in the amount of lean body mass and an increase in the amount of body fat[5].

The pancreas is an essential organ with both endocrine and exocrine tissues and plays an essential function in maintaining nutrient metabolism homeostasis in the body[6]. The accumulation of dysfunctional aging β cells with age can cause decreased glucose tolerance and diabetes[7]. Telomeres shortened by aging were reported to impair β cell function and participate in β cell destruction in the late stage of T2D[8]. The deletion of aging β cells in mouse models of type 1 diabetes showed increased insulin secretion and preserved insulin secretion ability, providing a link between cell aging and severe insulin deficiency[9].

In addition, considering that pancreatic weight, total insulin content, island size, and average insulin levels do not change, impaired signal transmission due to glucose stimulation during the aging process could be a decisive cause[10]. Some evidence suggested that the activation of inflammatory pathways contributed to insulin resistance in elderly people[11]. For example, aging is associated with inflammatory conditions in metabolic tissues and the upregulation of inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin-6 (IL-6), and IL-1 family members, which can directly interfere with insulin signaling pathways and cause metabolic dysfunction[12-14]. Aging toll-like receptor-4 deficient mice with reduced inflammatory responses showed decreased expressions of inflammatory markers and p16Ink4a (also known as CDKN2A) in adipose tissue and improved glucose tolerance compared to aging mice with intact inflammatory responses[15].

CAUSES OF SARCOPENIA DUE TO AGING

Muscles are the most necessary body components and play a pivotal role in maintaining a healthy life. Muscles are directly or indirectly related to muscle strength, energy, balance, and immunity. However, aging is a powerful vehicle for promoting sarcopenia[16,17]. It is known that basal metabolic rate decreases during the normal aging process. After the age of 30, it decreases at a rate of 3%-8% per

decade due to involuntary muscle loss. After the age of 50, approximately 1%-2% of muscle mass is lost per year. This rate increases to 3% per year after the age of 60, along with a decrease in strength of 1.5% annually[18,19].

Muscle loss has multiple factors, including lifestyle habits, disease triggers, and age-dependent biological changes. It is dealt with in the geriatric literature. However, it is starting to be studies into other areas dealing with the complexity of frail older persons. Testosterone levels gradually decrease with aging, and muscle protein synthesis and muscle mass can be reduced[20]. Growth hormone and insulin-like growth factor levels are also gradually and progressively decreased during normal aging. Such decreases are associated with decrease in muscle mass, not muscle strength[21,22].

The term sarcopenia was coined by Rosenberg[23] to describe the an age-related reduction in muscle mass that occurred with advancing age. However, muscle quality and structure are very important for each individual. V, and valid measurements are needed to establish the power of muscle mass[24]. Thus, sarcopenia that appears in elderly people and can be defined as the pathological loss of skeletal muscle[25]. It is characterized by structural changes in muscles along with that accompany dysfunction of muscles or decreased muscle strength. Sarcopenia should be considered a geriatric syndrome since multiple contributing factors (the aging process, diet, bed rest, sedentary lifestyle, chronic diseases, and drug treatment[26-28]) can cause the loss of muscle mass and that leads to an impaired state of health [29,30].

Sarcopenia has a multiple factorial origin[31]. Lifestyle habits, including physical inactivity, rest, and malnutrition, are known to can play an important role in most cases. In elderly people, changes in the endocrine system are, which is typical during the of aging process. They, can cause an imbalance between the anabolic process and the catabolic process[32], and a decreases of in anabolic hormones (testosterone, estrogens, growth hormone, insulin-like growth factor-1)[33], changes alterations of in the renin-angiotensin system[34], and vitamin D deficiency[35]. Low-grade systemic inflammation associated with, typical of aging and chronic disease, also plays an important role in increasing inflammatory cytokines.

RELATIONSHIP BETWEEN GLUCOSE METABOLIC AND EXERCISE

Glucose absorption by skeletal muscle contraction is caused by the presence of glucose transporter type 4 on the surface membrane and by accelerated diffusion according to the internal diffusion gradient for glucose[36]. Thus, the main step in controlling glucose absorption in skeletal muscles is the transport of glucose through cell membranes, and insulin and contractions induced *in vivo* by acute exercise or electrical stimulation can mediate glucose absorption in muscles[37].

Both aerobic exercise training and resistance exercise training are well known for their ability to restore systemic glucose homeostasis in people with metabolic T2D disease[38]. The relationship between glucose metabolism control and aerobic or resistance or combined exercise for both male and female pre-diabetes or diabetes patients are as follows. Twelve weeks of aerobic physical activity (60 min/d, 3 d/wk at 55%-65% HRR of rhythmic physical activity) and 12 wk of resistance physical activity (60 min/d, 3 d/wk at 55%-65% of 1 RM of machine weight) significantly decreased glycated hemoglobin (HbA1c) levels in pre-diabetes elderly people[39]; 12 wk of aquatic exercise (50 min/d, 3 d/wk at a rating on the perceived exertion scale of 10-16) improved glycemic control and decreased HbA1c in type 2 diabetes mellitus (T2DM) elderly people[40]; 6 mo of combined exercise (30 min of moderate aerobic exercise and 10 min of resistance exercise at 50%-70% of 1RM) significantly decreased HbA1c levels in T2DM elderly people[41]; 14 wk of resistance exercise (45 min/d, 3 d/wk at 60%-80% of 1RM for 1-8 wk and 70%-80% of 1RM for 10-14 wk) reduced plasma HbA1c levels and increased muscle glycogen stores in elderly people[42]; 2 years of aerobic exercise (60 min/d, 3 d/wk at 60%-70% of the HRmax) and resistance exercise (50 min/d, 3 d/wk of 13 types of resistance training protocols) HbA1C levels and β cell function were exercise responses in elderly patients with pre-diabetes[43]; 6 mo of resistance exercise (55 min/d, 3 d/wk at 75%-85% of 1 RM) was effective in improving glycemic control as shown by greater decreases in HbA1c levels[44]; 6 wk of high-intensity exercise training (3 d/wk supervised program at over 85% HRmax) increased insulin sensitivity in patients with T2DM[45]; 12 wk of 3 types of physical training (resistance, aerobic, and combined; 60 min/d, 3 d/wk) increased insulin receptor substrate (IRS)-1 expression by 65% in the resistance group and 90% in the combined group of patients with T2DM[46]; 8 wk of resistance and aerobic exercise (50 min/d, 2-3 d/wk at 65%-70% of 1RM and 65%-70% HRmax) significantly decreased HbA1c levels in both exercise groups[47], and 16 wk of low-intensity resistance training (2 d/wk at using body weight) significantly decreased HbA1c levels [48]. Nine studies contained elderly with T2DM are summarized the latest resistance exercises from traditional resistance exercises in Table 1.

EXERCISE FOR THE TREATMENT OF SARCOPENIA AND DIABETES

Sarcopenia is the age-related loss of skeletal muscle mass and strength that develops slowly over

Table 1 Resistance exercise and diabetes

Ref.	Study population and intervention	Study outcome
Kim *et al*[39], 2022	36 elderly people with pre-diabetics; 12 wk of resistance physical activity (60 min/d, 3 d/wk at 55%-65% of 1RM of machine weight)	Decreased glycated HbA1c levels
Nuttamonwarakul *et al*[40], 2012	20 elderly people with T2D; 12 wk of aquatic exercise (50 min/d, 3 d/wk at a perceived exertion (RPE) rating of 10-16)	Improved glycemic control and decreased HbA1c
Tan *et al*[41], 2012	25 elderly people with T2D; 6 mo of combined exercise (30 min of moderate aerobic exercise and 10 min of resistance exercise at 50%-70% of 1RM)	Decreased HbA1c levels
Castaneda *et al*[42], 2002	62 elderly patients with T2D; 14 wk of resistance exercise (45 min/d, 3 d/wk at 60%-80% of 1RM for 1-8 wk and 70%-80% of 1RM for 10-14 wk)	Reduced plasma glycosylated hemoglobin levels and increased muscle glycogen stores
He *et al*[43], 2022	82 elderly people with pre-diabetes; 2 years of resistance exercise (50 min/d, 3 d/wk of 13 types of resistance training protocols)	HbA1C levels and β cell function were resistance exercise response
Dunstan *et al*[44], 2002	36 elderly people with T2D; 6 mo of resistance exercise (55 min/d, 3 d/wk at 75%-85% of 1RM)	Improving glycemic control and decreases HbA1c levels
Jorge *et al*[46], 2011	48 middle-aged adults with T2D; 4 groups: Aerobic (n = 12), resistance (n = 12), combined (n = 12), and control (n = 12); 12 wk of training (60 min/d, 3 d/wk)	IRS-1 expression increased by 65% in the resistance group and by 90% in the combined group in T2DM
Ng *et al*[47], 2010	25 elderly people with T2D; 8 wk of resistance (50 min/d, 2-3 d/wk at 65%-70% of 1RM)	Decreased HbA1c levels
Takenami *et al*[48], 2019	10 elderly patients with T2D; 16 wk of low-intensity resistance training (2 d/wk at using body weight)	Decreased glycated hemoglobin

HbA1c: Hemoglobin; IRS: Insulin receptor substrate; T2DM: Type 2 diabetes mellitus.

decades and becomes an important factor in disability in the elderly population[49]. Insulin resistance in muscle protein metabolism with aging appears to be responsible for insensitivity to mixed supplements, and the presence of insulin resistance in muscle protein metabolism with aging independent of glucose tolerance has been demonstrated in healthy elderly subjects without diabetes[50]. Thus, the higher prevalence of sarcoidosis in T2DM individuals may be explained by other mechanisms, and the anabolic action of insulin in skeletal muscle is well known and may be progressively lost in T2DM due to decreased insulin sensitivity associated with the disease[51]. The decrease in muscle strength in elderly diabetes patients may be due, in part, to the intrinsic impairment of muscle strength generation, and a decrease in insulin signaling leads to a decrease in protein synthesis and an increase in proteolysis, which may ultimately lead to a decrease in muscle mass[52].

Resistance exercise is traditionally performed to increase muscle mass. Resistance exercise has a beneficial effect on sarcopenia in the general elderly population and is effective in coping with muscle mass reductions and performance deterioration in elderly patients with T2D[53,54]. Importantly, resistance exercise has also been found to have a beneficial effect on blood sugar profiles and insulin sensitivity[55]. In particular, in the case of elderly people, exercise is essential for preventing and managing sarcopenia because it counteracts the decline in both aging and muscle weakness caused by diabetes[56].

Compared to females who reported performing no strength training, females who performed strength training showed a 30% reduction in T2D (hazard ratio = 0.70, 95% confidence interval: 0.61-0.80)[57]. Short-term acute (2 d) moderate-intensity resistance exercise (50% of 1 RM) effectively reduced blood glucose levels and blood glucose fluctuations in elderly patients with T2M and sarcopenia[58]. Table 2 summarizes the benefit of resistance exercise in elderly people with sarcopenia.

Aging can accelerate the loss of muscle mass and function, and the loss of muscle mass and function may impair glucose metabolism and aggravate diabetes[59]. For this reason, elderly people especially, need to increase muscle mass, and the only way to increase muscle mass is to perform resistance exercises. The inclusion of gradual resistance exercise in lifestyle modification programs should be considered for elderly patients with sarcopenia and T2D or both[58,60]. There is also a general consensus that a moderate increase in daily protein intake to 0.8 g/kg/d or more in elderly people may enhance the metabolism of muscle proteins and reduce the progressive loss of muscle mass with aging [61].

CONCLUSION

Among the various diseases caused by aging, diabetes and sarcopenia appear in elderly people. Reduced β cell function in elderly people lowers insulin sensitivity, which affects protein synthesis and

Table 2 Resistance exercise and sarcopenia

Ref.	Study population and intervention	Study outcome
Zhao *et al*[58], 2022	24 elderly patients with T2D and sarcopenia; short-term acute resistance exercise (40 min/d, 3 d at 50% of 1RM)	Decreased blood glucose levels, blood glucose fluctuations and the risk of hypoglycemia
Seo *et al*[62], 2021	12 elderly females with sarcopenia; 16 wk of resistance training (60 min/d, 3 d/wk at 4-8 on the OMNI scale)	Improved functional fitness and muscle quality
Dong *et al*[63], 2019	21 elderly patients on maintenance hemodialysis with sarcopenia; 12 wk of resistance exercise (3 d/wk at their own body weight and elastic balls)	Improved physical activity status (maximum grip strength, daily pace, and physical activity level), and Inflammatory factors (IL-6, IL-10, and TNF-α)
Liao *et al*[64], 2018	56 elderly females with sarcopenia obesity; 12 wk of elastic band resistance training (3 training sessions every week for 12 wk, each training session was performed for 55 min)	Significant beneficial effect on muscle mass, muscle quality, and physical function
Hamaguchi *et al*[65], 2017	7 elderly females with sarcopenia; 6 wk of progressive power training (2 sessions per week for 6 wk; when the subject was capable of completing all 8 sets, the weight was increased by 380-760 g in the next session)	BMD and knee extensor strength were significantly greater in the training group than in the control group
Vasconcelos *et al*[66], 2016	14 elderly females with sarcopenia; 10 wk of resistance exercise (60 min/d, 2 d/wk; 1-2 wk at 50% of 1RM, 3-4 wk at 75% of 1RM, 5-6 wk at 40% of new 1RM, and 7-10 wk at 60% of new 1RM)	Knee extensor power was significantly higher in the training group than in the control group
Stoever *et al*[67], 2018	28 elderly people with sarcopenia obesity; 16 wk of progressive resistance training (2 d/wk, increasing to 80%-85% of maximum strength with 3 sets of 8 to 12 repetitions)	Increase performance in hand-grip strength, gait speed, SPPB score, and modified PPT score

T2D: Type 2 diabetes; IL: Interleukin; TNF: Tumor necrosis factor; SPPB: Short Physical Performance Battery; PPT: Physical performance test.

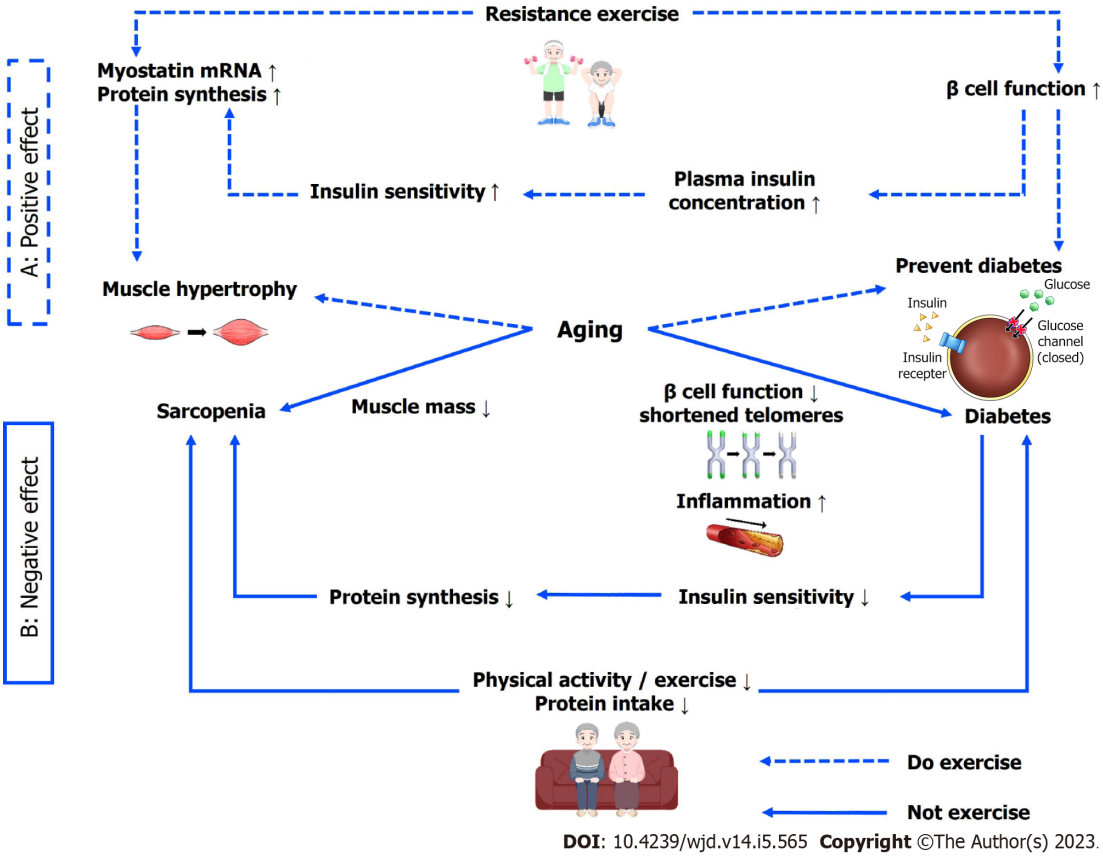

Figure 1 The summary of the factors that cause diabetes and sarcopenia due to the aging and benefits of resistance exercise in the elderly is as follows.

interferes with muscle synthesis. The functional decrease and aggravation of disease in elderly people with less regular exercise or physical activity causes imbalances in food intake and a continuous, vicious cycle. In contrast, resistance exercise increases β cell function and protein synthesis in elderly people. A summary of our conclusions is shown in Figure 1. Regular physical activity and/or resistance exercise

in the elderly is effective in preventing and promoting sarcopenia and diabetes. On the contrary, aging increases the risk of exposure to sarcopenia and diabetes. Therefore, exercise or physical activity should be regularly performed even before aging begins, and muscle mass should be increased through resistance exercise. The protein intake necessary for protein synthesis during resistance exercise should also be maintained in elderly people and those with diabetes or/and sarcopenia.

FOOTNOTES

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MINIREVIEWS

Intermediate hyperglycemia in early pregnancy: A South Asian perspective

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Abstract

"Intermediate hyperglycemia in early pregnancy (IHEP)" refers to mild hyperglycemia detected before 24 gestational weeks (GW), satisfying the criteria for the diagnosis of gestational diabetes mellitus. Many professional bodies recommend routine screening for "overt diabetes" in early pregnancy, which identifies a significant number of women with mild hyperglycemia of undetermined significance. A literature search revealed that one-third of GDM women in South Asian countries are diagnosed before the conventional screening period of 24 GW to 28 GW; hence, they belong in the IHEP category. Most hospitals in this region diagnose IHEP by oral glucose tolerance test (OGTT) using the same criteria used for GDM diagnosis after 24 GW. There is some evidence to suggest that South Asian women with IHEP are more prone to adverse pregnancy events than women with a diagnosis of GDM after 24 GW, but this observation needs to be proven by randomized control trials. Fasting plasma glucose is a reliable screening test for GDM that can obviate the need for OGTT for GDM diagnosis among 50% of South Asian pregnant women. HbA1c in the first trimester predicts GDM in later pregnancy, but it is not a reliable test for IHEP diagnosis. There is evidence to suggest that HbA1c in the first trimester is an independent risk factor for several adverse pregnancy events. Further research to identify the pathogenetic mechanisms behind the fetal and maternal effects of IHEP is strongly recommended.

Key Words: Intermediate hyperglycemia; Early pregnancy; Gestational diabetes; South Asian women; Adverse events; Asian Indian

Core Tip: Intermediate hyperglycemia in early pregnancy (IHEP) is a common metabolic disorder among South Asian pregnant women, and it accounts for one-third of women with "gestational diabetes mellitus". The benefits of early therapeutic intervention for these women have not been established. The guidelines on the screening and management of IHEP by international and regional professional bodies are conflicting, producing major confusion in obstetric practice in South Asian countries. There is an urgent need for randomized controlled trials to settle the ongoing controversies in this field.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is the most common metabolic abnormality in pregnancy, and its prevalence varies widely depending on the population studied and the diagnostic strategy employed. GDM predisposes pregnant women to several obstetric and perinatal complications and places the mother and infant at high risk of long-term metabolic morbidity[1-3]. For many years, GDM was defined as "any degree of glucose intolerance that was first recognized during pregnancy"[4]. However, this definition fails to distinguish between women with "new onset of glucose intolerance in pregnancy" and those with preexisting undiagnosed diabetes. To circumvent this diagnostic confusion, the World Health Organization (WHO 2013) introduced the broad term hyperglycemia in pregnancy (HIP) for various dysglycemias in pregnancy[5]. Furthermore, women with HIP are subcategorized into two distinct entities: (1) Diabetes in pregnancy (DIP), those women satisfying the WHO (2006) diagnostic criteria of diabetes in a nonpregnant state (undiagnosed preexisting diabetes); and (2) GDM, women having plasma glucose values in a 75 g oral glucose tolerance test (OGTT) above the threshold values proposed by the International Association of DIP Study group (IADPSG) criteria[6] and below the threshold for diagnosis of overt diabetes at any stage of pregnancy. Screening for DIP at the first prenatal visit is accepted by several preeminent organizations, such as the International Federation of Gynecology and Obstetrics (FIGO)[7], the International Diabetes Federation (IDF)[8] and the American Diabetes Association (ADA)[4]. In contrast, the screening and diagnosis of GDM continue to be controversial. Although OGTT is generally accepted as the diagnostic test by several professional organizations, there is no agreement on the glucose load for the test, plasma glucose cut off values and the number of abnormal plasma glucose values required for GDM diagnosis. Furthermore, there is no international consensus on GDM screening strategies: Risk-based selective or universal screening, one-step or two-step screening and optimal timing of screening (Table 1).

Conventionally, GDM screening is performed between 24-28 wk of gestation (GW). The selection of this period is justified by: (1) The development of significant physiological insulin resistance by 24 GW; and (2) the availability of sufficient time in pregnancy for therapeutic intervention after GDM diagnosis. The GDM criteria proposed by O'Sullivan and Mahan[9] and subsequently modified by Carpenter and Coustan[10] were used to identify pregnant women who are prone to type 2 diabetes later in life. These criteria and the subsequent WHO 1999 criteria[11] were not validated by any obstetric or perinatal outcome studies. The landmark Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study revealed a continuous relationship between maternal glycemia between 24 wk and 32 wk and several pregnancy adverse events, which formed the basis of the glucose threshold values proposed in the IADPSG criteria[6,12]. The threshold values of the IADPSG criteria are widely accepted by several professional organizations for GDM diagnosis between 24 GW and 28 GW[5,7,8]. However, the American College of Obstetricians and Gynecologists (ACOG)[13] and National Institute for Health and Care Excellence (NICE)[14] follow different criteria for GDM diagnosis. Many countries in South Asia continue to follow modified WHO 1999 criteria to suit the behavior of their obstetric population: DIPSI criteria[15] (Table 1).

GDM diagnosis prior to 24 GW (early GDM) by any criteria is not validated by pregnancy outcome data. Despite this limitation, many professional bodies, such as the WHO, FIGO, ACOG, and Australasian DIP Society (ADIPS), continue to recommend screening for early GDM among high-risk population groups[5,7,13,16] (Table 2). However, many organizations question the validity of mild hyperglycemia detected in early pregnancy. In 2016, the IADPSG withdrew its earlier 2010 recommendation to diagnose GDM in early pregnancy based on an abnormal fasting plasma glucose (FPG) value of ≥ 5.1 mmol/L[17]. The 2021 United States Preventive Services Task Force statement concluded that 'the current evidence is insufficient to assess the balance of benefits and harms of screening for GDM before 24 GW[18]. The NICE guidelines (2021) restrict GDM screening in early pregnancy to women who had GDM in a previous pregnancy[14]. The ADA 2022 limits "GDM" terminology to

Table 1 Commonly used oral glucose tolerance test criteria for gestational diabetes diagnosis among South Asian women

Criteria	Glucose load	Plasma glucose threshold values				Number of abnormal values required for diagnosis	Remarks
		FPG mmol/l	1 h PG mmol/L	2 h PG mmol/L	3 h PG mmol/L		
IADPSG, WHO 2013, ADA proposed "One step" procedure	75 g	≥ 5.1	≥ 10.0	≥ 8.5	-	1	Universal screening
DIPSI	75 g	-	-	≥ 7.8	-	1	Universal screening. OGTT in non-fasting state
ACOG and ADA proposed "Two step" procedures							
Carpenter and coustan criteria	100 g	≥ 5.3	≥ 10.0	≥ 8.6	≥ 7.8	2	Universal screening, prior 50 g GCT positivity required (1-h PG ≥ 7.2 or 2-h PG ≥ 7.8 mmol/L)
NDDG criteria	100 g	≥ 5.8	≥ 10.5	≥ 9.0	≥ 8.0	2	
						ACOG (2018) acknowledges higher risk for those with one abnormal value	ACOG (2018) permits institutions and individuals to use one step IADPSG procedure as well
NICE	75 g	≥ 5.6	-	≥ 7.8	-	≥ 1	Selective testing for high risk population[1]

[1]High risk population = women having Body Mass Index > 30 kg/m^2, previous macrosomia (≥ 4500 g, previous GDM, family history of diabetes, ethnic origin with high prevalence of diabetes (South Asian, Black Caribbean, and Middle Eastern).

IADPSG: International Association of the Diabetes and Pregnancy Study Groups; WHO: World Health Organization; ADA: American Diabetes Association; DIPSI: Diabetes in Pregnancy Study group of India; ACOG: American College of Obstetricians and Gynaecologists; NICE: National Institute for Health and Care Excellence; OGTT: Oral glucose tolerance test; OCT: Oral glucose challenge test; NDDG: National Diabetes Data Group; FPG: Fasting plasma glucose; PG: Post load plasma glucose.

denote impaired glucose tolerance detected in the second and third trimesters only[4]. However, it recommends screening before 15 GW to identify: (1) Undiagnosed pregestational diabetes; and (2) women at risk for adverse events, *i.e.*, those with FPG ≥ 6.1 mmol/mol or HbA1c ≥ 41 mmol/mol (Table 2).

The common practice of early GDM screening (before 24 GW) and DIP screening at the first prenatal visit among high-risk pregnant women identifies many women with milder glucose intolerance of undetermined significance: Glycemia below the threshold for overt diabetes but satisfying the diagnostic criteria for GDM. This dysglycemia in early pregnancy (before 24 GW) is referred to as Intermediate Hyperglycemia in Early Pregnancy (IHEP) and forms a significant proportion of "GDM women" in South Asian countries (India, Pakistan, Bangladesh, Sri Lanka, Nepal). This article is an update on the current knowledge on IHEP among pregnant women residing in South Asian countries.

SOUTH ASIANS AS A DIABETES RISK POPULATION

South Asians represent approximately 2 billion people globally. A high prevalence of type 2 diabetes has been reported among South Asians residing in the Indian subcontinent as well as in its diaspora [19]. The clinical profile of type 2 diabetes among South Asians differs from that among Caucasians in various aspects: Onset at a younger age, lower body mass index (BMI), higher abdominal (visceral) obesity, greater insulin resistance and early decline in pancreatic β cell function[20]. There is an ongoing global epidemic of type 2 diabetes with its epicenter in South Asia, and India is being projected as the "diabetic capital" of the world. The number of people with diabetes in India has increased exponentially in the past two to three decades: 19 million in 1995, 32 million in 2000, and 66.8 million in 2014, and this number is expected to increase to 79.4 million in 2025[20,21].

The ICMR-INDIa DIABetes (INDIAB) study revealed that the number of people with prediabetes (77.2 million) in India was higher than that of people with diabetes (62.4 million)[22]. The IDF estimated 76 million women aged 20 years to 39 years to have diabetes or prediabetes in the Asia-Pacific region [23]. The high prevalence of prediabetes among women of child-bearing age is mirrored by the high GDM prevalence in pregnancy in this region. India has 5.7 million women with hyperglycemia during pregnancy and ranks first in the world in this respect[8,24]. A similar higher propensity for GDM has been reported among Asian immigrants in developed countries. Asian immigrants in the United Kingdom and Norway (South, East, and West Asian immigrants) have double the odds for GDM than

Table 2 Recommendations of various organizations for "intermediate hyperglycemia" screening before 24 gestational weeks

Organization	Timing	Target population	Test	Threshold PG values in mmol/L	Position of the association in 2022
International Association of the Diabetes and Pregnancy Study Group (IADPSG): 2010	First antenatal visit	Universal or only high-risk women	Fasting plasma glucose	5.1-6.9; if < 5.1, OGTT after 24 GW	2016: Withdrew the recommendation for FPG testing before 24 GW
World Health Organization: 2013	Any time before 24 GW	Not defined	75 g OGTT	FPG 5.1-6.9; 1-h PG ≥ 10; 2-h PG 8.5-11.0	No change from 2013 recommendation
American Diabetes Association (ADA): 2010	During first antenatal visit, suggest risk stratification	Those women with marked obesity, personal history of GDM, glycosuria, or a strong family history of diabetes, testing as soon as possible	One step test: 75 g 2-h OGTT, or two step test: 50 g OCT + 100 g 3-h OGTT	One step: FPG ≥ 5.2, 1-h ≥ 10, 2-h ≥ 8.6 (one abnormal value); two step: FPG ≥ 5.2, 1-h ≥ 10.0, 2-h ≥ 8.6, 3-h 7.8 (require two abnormal values)	2015: Test for undiagnosed diabetes at the first prenatal visit for those with risk factors, using standard diagnostic criteria; 2021: Test for undiagnosed pre-diabetes and diabetes at the first prenatal visit in those with risk factors using standard diagnostic criteria; 2022: Before 15 GW, test women with risk factors or consider testing all women for undiagnosed DM
2011-2014: Accepted IADPSG criteria for GDM diagnosis at 24-28 GW	No guideline for screening before 24 GW	Not specified	Nil		Screen women at risk for adverse events by FPG (6.1 mmol/L), HbA1c (4.1 mmol/mol)
American College of Obstetricians and Gynaecologists (ACOG): 2018	First antenatal visit, selective for women at risk for undiagnosed diabetes and GDM	Selective for women at risk for undiagnosed type 2 diabetes or GDM	Two step: 50 g OCT + 100 g 3-h OGTT[1] or one step: 75 g OGTT in select situations	FPG > 5.3, > 5.8; 1-h PG ≥ 10, ≥ 10.6; 2-h PG ≥ 8.6, ≥ 9.2; 3-PG ≥ 7.8, ≥ 8.0 (NDDG or C&C criteria); one step same as IADPSG recommendations for DM, no specific recommendation for intermediate hyperglycemia	No Changes in criteria after 2018
Diabetes In Pregnancy Study group of India (DIPSI)	Yes	Universal	Non fasting 75 g OGTT	2 h PG ≥ 7.8	No further modifications
National Institute for Health and Care Excellence (NICE): 2015 and 2021	Yes	Selective for women with history of previous GDM at first antenatal visit; other risk factors, no testing before 24 GW	Blood self-monitoring of glucose or 75 g OGTT	FPG ≥ 5.6; 2 h PG ≥ 7.8	No further modification

[1]American College of Obstetricians and Gynecologists approves both Carpenter and Coustan criteria and National Diabetes Data Group and PG threshold values of both criteria are shown.

3-PG: 3 h post glucose load plasma glucose values; GW: Gestational week; OGTT: Oral glucose tolerance test; PG: Plasma glucose; FPG: Fasting plasma glucose; DM: Type 2 diabetes; GDM: Gestational diabetes; OCT: Oral glucose challenge.

non-Hispanic whites residing in these countries[25]. In a recent analysis by Gami *et al*[26] among the United States population, GDM rates increased significantly from 47.6 to 63.5 per 1000 live births from 2011 to 2019, and this rise was mainly observed among Asian Indian and Puerto Rican women. Additionally, women of Asian ancestry in the United States were observed to have GDM at a younger age, even with BMI within or below the normal range[27,28]. In a large study involving 10353 pregnancies at Bradford Infirmary in the United Kingdom, Farrar *et al*[29] estimated that the glucose threshold levels in a 75 g OGTT (performed between 26-28 GW) produced a 75% or higher relative risk of large for gestational age (LGA) babies among South Asian women than among British Caucasian women. The plasma glucose threshold values for LGA babies among South Asian and British Caucasian women were FPG values of 5.2 mmol/L and 5.4 mmol/L, respectively, and 2-h post glucose load plasma glucose (2-h PG) values of 7.2 mmol/L and 7.5 mmol/L, respectively.

IHEP AMONG SOUTH ASIAN PREGNANT WOMEN

The screening strategies to identify IHEP/HIP are: (1) Universal or selective screening by OGTT; (2) FPG at the first prenatal visit; and (3) hemoglobin A1c (HbA1c) in early pregnancy. We performed a

literature search for studies carried out between January 2004 and November 2022 on "IHEP among women residing in South Asian countries" in PubMed (medline), Cochrane Library and Google Search using the terms "gestational diabetes mellitus", "diabetes in pregnancy", "hyperglycemia in pregnancy", "early diagnosis", "first trimester", "early pregnancy", "South Asia", "India", "HbA1c", "oral glucose tolerance test", "fasting glucose", and "intermediate hyperglycemia". We identified 19 original articles that provided data on the frequency of IHEP in the South Asian region. These studies were not primarily designed to assess IHEP (early GDM) and had inadequate data for a proper systematic review or meta-analysis on this topic.

OGTT for detection of IHEP

The literature search yielded 14 GDM studies from South Asia with some data on the frequency of IHEP: Eleven from India, two from Sri Lanka and one from Bangladesh. The study design, GDM diagnostic criteria, overall GDM prevalence and frequency of IHEP in these studies are shown in Table 3 [30-43]. The marked heterogeneity in the study design, the diversity of the GDM diagnostic criteria and the lack of clinical details of women with IHEP are limitations to making a comparative assessment between these studies. Five GDM diagnostic criteria were used in these studies: WHO 1999 criteria for six studies (4 studies[31,36,41,43] using both fasting PG and 2-h PG values, 2 studies[32,33] using only 2-h PG value; modified WHO 1999 criteria), DIPSI criteria for four studies (same as modified WHO 1999 criteria, but OGTT performed in nonfasting state)[34,37,38,40], IADPSG criteria for three studies[30,39,42] and Carpenter & Coustan criteria for one study[35]. As WHO-1999, modified WHO 1999 and DIPSI criteria are primarily based on 2-h PG values, the women who had GDM diagnosis by these criteria were analyzed together. The pooled data analysis of 32055 pregnant women who were screened by these criteria revealed that 4024 women had GDM, with a prevalence of 12.55%. Of 4006 women who were screened by IADPSG criteria, 1072 women had GDM, with a prevalence of 26.75%. One small study among 298 women identified 40 GDM by Carpenter & Coustan criteria, with a prevalence of 13.42%.

The number of women with GDM in different periods of gestation and their percentage in relation to total GDM women are shown in Table 3. The pooled data analysis revealed that 925 (18.5%) of 4961 GDM women in eleven studies had a GDM diagnosis in the first trimester. The combined data of seven studies showed that 1230 (32.6%) of 4961 GDM women were diagnosed before the conventional screening period of 24-28 GW. Hence, one-third of GDM women in South Asian countries belong to the IHEP category, and half of them are diagnosed in the first trimester. A selective assessment of women with IHEP diagnosis by IADPSG criteria (data from 3 studies)[30,39,42] revealed nearly the same proportions of women with IHEP in the first trimester (18.09%) and < 24 GW (35.31%) groups. The exclusion of women with DIP from the analysis[39,41,43] produced minor changes in the frequency of IHEP: First trimester, 19.55% (149 of 762 GDM women); before 24 GW, 31.03% (359 of 1157 GDM women).

The above data suggest that OGTT is widely used for the detection of IHEP among South Asian women. The Ministry of Health and Family Welfare, Government of India Technical Guideline on the Diagnosis of Gestational Diabetes (2018), recommends that all pregnant women should undergo 75 g OGTT "during the first antenatal contact as early as possible"; if the test is negative initially, a second OGTT should be done during 24-28 GW[44-46]. The FIGO endorsed this approach for hyperglycemia screening in early pregnancy in South Asian countries[7]. Similarly, the ACOG[13], ADIPS[16], and Canadian Diabetes Association[47] advocate OGTT-based screening for IHEP among the South Asian diaspora in the respective countries.

There is no consensus on the OGTT criteria to be used for IHEP diagnosis in the South Asian region (Table 2). Considering the convenience of nonfasting state and single PG sampling, the DIPSI criteria are frequently used in India for "GDM diagnosis" in all trimesters[32]. However, there are some concerns about the validity of DIPSI criteria in the post-IADPSG era. The DIPSI 2-h PG threshold value (7.8 mmol/L) was derived from WHO 1999 criteria, a popular criteria for GDM diagnosis during the 1999-2010 period[11]. The FPG threshold value of 7 mmol/L recommended for GDM diagnosis in the WHO 1999 criteria is presently the cut off value for DIP diagnosis, and women with DIP are not considered to have GDM by any professional organization. Furthermore, with the introduction of IADPSG criteria based on the pregnancy outcome data in the HAPO study, the WHO withdrew its 1999 criteria and recommended IAPDPG criteria as the new WHO2013 criteria[5]. The DIPSI criteria were initially validated with WHO 1999 criteria, and many hospitals in India continue to use these criteria for GDM diagnosis[33] (Table 2). However, as the WHO has withdrawn its 1999 criteria and accepted the IADPSG criteria, the DIPSI criteria need to be revalidated with the WHO 2013 criteria or be validated by pregnancy outcome data. The validation of nonfasting DIPSI criteria with IADPSG criteria was attempted in two well-designed studies from India; in both studies, the sensitivity for DIPSI criteria was too low for its use as a diagnostic or screening test for GDM[48,49].

FPG estimation for detection of IHEP

In the HAPO study on which the IADPSG criteria are based, there was heterogeneity in the frequency of abnormal FPG, 1-h PG and 2-h PG values among women diagnosed with GDM in different centers. An abnormal FPG value occurred only in 26% of women in the Hong Kong center, while the percentage in

Table 3 Early Gestational diabetes among South Asian women: Oral glucose tolerance test based studies

Ref.	Region study location	No. of women	Diagnostic criteria and study design	GDM women-*n* (prevalence %), GW, no of GDM women (% of total GDM women)
Sharma *et al*[36], 2013	Jammu, India hospital	500	WHO 1999; preceded by 75 g non fasting OCT if 2-h PG ≥ 7.8 mmol/L, 75 g OGTT; at first prenatal visit	GDM: *n* = 55 (10%); **16-20 wk, 10 (18.1%); 21-24 wk, 20 (36.3%);** 25-28 wk, 10 (18.1%); 29-32 wk, 15 (27.2%)
Seshiah *et al*[31], 2008	Chennai, Indian community	12056	WHO 1999; test at first prenatal visit; repeat at 24 GW and 32 GW	GDM: *n* = 1679 (13.9%); **< 16 wk, 208 (12.4%); 17-23 wk, 280 (23.0%);** ≥ 24 wk, 891.0%-64.6%
Dahiya *et al*[34], 2014	Rohtak, India hospital	500	DIPSI; test < 16 GW, if negative repeat at 24-28 GW	GDM: *n* = 35 (7%); **< 16 wk, 4 (11.4%);** second trimester-34 (88.6%)
Veeraswamy *et al* [37], 2016	Pan India study; peripheral clinic	9282	DIPSI; OGTT at first prenatal visit	GDM: *n* = 740 (8%); **1st trimester, 233 (31.5%);** 2nd trimester, 320 (43.2%); 3rd trimester, 187(25.3%)
Neelakandan *et al* [30], 2014	Tirucharapalli, India hospital	1106	IADPSG; if preceding by 50 g OCT 1-h PG ≥ 7.2 mmol/L; first prenatal visit	GDM: *n* = 258 (23.3%); **≤ 12 wk, 36 (13.9%); 13-18 wk, 43 (16.7%);** 19-28 wk, 114 (44.1%); ≥ 28 wk, 65 (25.2%)
Bhatt *et al*[38], 2015	Pune, India community	989	DIPSI with Capillary Glucose; OGTT any trimesters	GDM: *n* = 88 (8.9%); **< 24 wk, 42 (47.9%);** ≥ 24 wk, 46 (52.1%)
Anjalakshi *et al*[33], 2009	Chennai, India hospital	800	WHO 1999-M; OGTT between 16-32 GW	GDM: *n* = 87 (10.89%); **16-20 wk, 7 (8%); 21-24 wk, 17 (19.5%);** 25-28 wk, 49 (56.3%); 29-32 wk, 14 (16.1%)
Seshiah *et al*[32], 2007	Chennai, India community	4151	WHO 1999-M; any trimester	GDM: *n* = 741 (17.9%); **< 16 wk, 121 (16.3%); 17-23 wk, 166 (22.4%);** ≥ 24 wk, 454 (61.27%)
Grewal *et al*[32], 2007	Delhi, India hospital	298	Carpenter and Coustan criteria; OGTT before 12 GW; women with DIP, IFG, and IGT excluded	GDM: *n* = 40 (13.42%); **< 12 wk, 24 (60%);** 24-28 wk, 16 (40%)
Bahl *et al*[40], 2022	Delhli, India community	2244	DIPSI; OGTT at first prenatal visit, repeat 24-28 wk, 34-36 wk	GDM: *n* = 430 (19.16%); **1st trimester, 112 (26.1%);** 2nd and 3rd trimester, 318 (74%)
Punnose *et al*[39], 2023	Delhi, India hospital based	2638	IADPSG; first trimester HbA1c, if < 48 mmol/L, OGTT at any trimester; if OGTT negative before 24 GW repeat after 24 GW, DIP excluded	GDM: *n* = 722 (27.37%); **< 14 wk, 125 (17.3%); 14-23 wk, 130 (18%);** ≥ 24 wk, 467 (64.68%)
Sudasinghe *et al*[43], 2016	SriLanka community	1533	WHO-1999; initial screening in first trimester by 2-h post prandial PG ≥ 6.7-11.1 mmol/L OGTT at 16 GW, if negative repeat after 24 GW, DIP excluded	GDM: *n* = 169 (11.02%); **< 16 wk, 19 (12.67%)**
Jayawardane *et al* [41], 2018	Sri Lanka hospital	Not given	WHO 1999 (2011-14) and DIPSI (2014-15), OGTT in any trimesters, DIP excluded	GDM: *n* = 435 (total number not available); **12-23 wk, 104 (23.9%);** ≥ 24 wk, 331 (76.09%)
Mazumder *et al*[42], 2022	Bangladesh community	265	IADPSG; OGTT in any trimester	GDM: *n* = 92 (34.71%); **1st trimester, 33 (35.87%);** 2nd trimester, 36 (39.13%); 3rd trimester, 23 (25%)

Values in bold indicate percentage of women diagnosed before 24 gestational weeks. GW: Gestational weeks; WHO: World Health Association; OCT: Oral glucose challenge; OGTT: Oral glucose tolerance test; DIPSI: Diabetes In Pregnancy Study group of India; IADPSG: International Association of the Diabetes and Pregnancy Study Group; DIP: Diabetes in pregnancy; IGT: Impaired glucose tolerance; IFG: Impaired glucose tolerance; 1-h PG: Post Glucose load 1 h plasma glucose.

the Bellflower (California) center was > 70%. This observation led to the conclusion that FPG performed poorly in diagnosing GDM in Asians compared to Caucasian women[50]. A study in South India by Balaji *et al*[51] also suggested that only 24% of women who had GDM diagnosis by WHO 1999 criteria had FPG values ≥ 5.1 mmol/L (the IADPSG FPG threshold for GDM diagnosis), and the authors concluded that FPG was inadequate to diagnose GDM in the South Asian population. However, the reliability of FPG to diagnose GDM by IADPSG criteria (at least based on the available FPG and 2-h PG values) was not assessed in this paper. Subsequently, several studies among South Asian pregnant women reported FPG as a more reliable, easier test than the glucose challenge test to screen for GDM by Carpenter and Coustan criteria[52-54]. In a large North Indian study (involving 6520 pregnant women), an FPG value of ≤ 4.3 mmol/L reliably ruled out GDM in all trimesters (95.6% sensitivity), and FPG

alone (≥ 5.1 mmol/L) could identify 67.9% of GDM by IADPSG criteria[55]. This study suggested that FPG can reliably "rule in and rule out GDM" and can avoid OGTT for GDM diagnosis in approximately 50% of South Asian pregnant women. The excellent area under the curve of 0.909 (95%CI: 0.898 to 0.920) for FPG in this study was contrary to the traditional belief that FPG performs poorly as a screening test for GDM in Asians.

Several studies from South Asia have tested the reliability of FPG in early pregnancy to predict GDM in later pregnancy. In a cohort of 246 pregnant women from North India, an FPG value of 4.7 mmol/L in early pregnancy reliably predicted GDM diagnosis by IADPSG criteria after 24 GW (with 94% sensitivity and 74% specificity)[56]. Another study from South India (n = 270) concluded that FPG ≥ 5 mmol/L in the first trimester reliably predicted GDM by DIPSI criteria, with an area under the ROC curve of 0.694, sensitivity of 86.6%, and specificity of 52.1%[57].

The above data suggest that FPG estimation in early pregnancy can be a reliable predictor and possibly a screening test for GDM among South Asian pregnant women. In 2013, most international professional organizations accepted the IADPSG recommendation to diagnose GDM in early pregnancy based on FPG values between 5.1 and 6.9 mmol/L[5,7,8]. Subsequently, IADPSG withdrew this recommendation[17], and some organizations supported this change[4]. Presently, FPG values between 5.1 and 6.9 mmol/L in early pregnancy are interpreted differently by many professional bodies. The WHO approves GDM diagnosis for such women and permits treatment accordingly. The IADPSG does not approve FPG use for GDM diagnosis before 20 GW. The ADA (2022) criteria approve treatment for these women, provided the FPG is ≥ 6.1 mmol/L and it is documented before 15 GW. The DIPSI and Government of India (2018) guidelines do not recommend FPG estimation at any stage of pregnancy. Obstetricians in South Asian countries follow all these guidelines, resulting in chaos in the diagnosis and management of IHEP among South Asian women.

HbA1c for detection of IHEP

Following the recommendation of the World Health Organization that HbA1c testing be used for the diagnosis of diabetes mellitus in the general population, interest in its use in pregnancy has been renewed[58]. An HbA1c level ≥ 48 mmol at booking is now accepted as a criterion to diagnose DIP or preexisting overt diabetes[5,7]. In 2011, the California state Diabetes and Pregnancy program (CSDPP) "Sweet Success" adopted a new algorithm for the diagnosis and treatment of hyperglycemia in pregnancy[59]. Accordingly, all women with HbA1c values of 39-46 mmol/mol in early pregnancy are advised to undergo GDM treatment without further confirmatory OGTT. This recommendation equates GDM to the prediabetic state of the nonobstetric population. This CSDPP proposal, although practiced in several United States centers, has not been approved by any professional body.

Considering the high prevalence of prediabetes in the background population, HbA1c can be a potential biomarker to identify high GDM risk women in early pregnancy among South Asian Women. There are limited studies among South Asian women to assess HbA1c as a diagnostic test for IHEP. In a South Indian study to assess HbA1c for screening GDM among 507 women by Balaji *et al*[60], a subgroup analysis revealed that all women with HbA1c ≥ 42 mmol/mol in the first trimester (n = 10) developed GDM (by WHO 1999 criteria) in later pregnancy. In another study in which HbA1c and OGTT were simultaneously tested at a mean age of 19 GW, women who had GDM had higher HbA1c (33 mmol/mol) than those without GDM (HbA1c, 30 mmol/mol)[61]. In a retrospective cohort study from our center among 2275 Asian Indian pregnant women, an HbA1c value of > 37 mmol/mol in the first trimester was found to be an independent predictor of GDM (adjusted OR 2.60, 95%CI: 1.49-4.55) by IADPSG criteria[62]. However, HbA1c in the first trimester lacked sufficient sensitivity and specificity for consideration as a diagnostic test for GDM in early pregnancy. Interestingly, we observed in this cohort that, even after exclusion of women with DIP and women who developed GDM in later pregnancy, HbA1c in the first trimester was independently associated with preterm birth and primary cesarean delivery[63]. Hence, apart from being a strong risk factor for GDM, HbA1c in the first trimester can independently predict adverse pregnancy events in South Asian pregnant women.

As HbA1c is increasingly being used to identify DIP at the first prenatal visit, it is cost effective to use the same test for the prediction of GDM and other adverse events. Furthermore, HbA1c estimation requires only a single nonfasting sample, and the test has greater preanalytic stability and reproducibility and no interference from acute stressful conditions. These factors are of special advantage for pregnant women in South Asian countries, as most of them report to hospitals in a nonfasting state and are not willing to undergo repeated blood sampling[15].

INTERVENTIONS AMONG SOUTH ASIAN WOMEN WITH IHEP

Limited data on IHEP management in South Asian countries are derived from the analysis of retrospective data. With early initiation of treatment among a small cohort of 54 women with early GDM (by WHO 1999 criteria) in South India, the birth weight of babies of GDM women was comparable to babies of non-GDM women[64]. In a retrospective study in our center among 2638 pregnant women with HbA1c < 48 mmol/mol in the first trimester, 255 women satisfied the IADPSG criteria for GDM

before 24 GW (IHEP)[39]. Despite early initiation of treatment, women with early GDM (IHEP) had significantly higher adjusted odds ratios for premature birth, macrosomia, LGA babies, and neonatal intensive care unit admission and lower odds for normal vaginal delivery than non-GDM women. The highest risk for adverse events was observed among GDM women who had the diagnosis in the first trimester. A similar observation was made in a large multiethnic Australian study that revealed the highest adverse events among women who had GDM diagnosis in the first trimester, despite the best practices of management[65]. The failure to reduce adverse pregnancy events by early intervention in these studies[39,65] may be interpreted as a lack of benefit of early GDM screening. Alternatively, it can be attributed to the fetal and maternal effects of mild hyperglycemia in early pregnancy, which were not reversed with restoration of euglycemia in later pregnancy. This speculation is strengthened by the observation of an independent association of HbA1c in the first trimester with adverse events, even without the development of GDM in later pregnancy by several researchers[63,66,67].

IHEP AMONG SOUTH ASIAN PREGNANT WOMEN: CHALLENGES & RECOMMENDATIONS

The main challenges in the identification and management of IHEP are the lack of pregnancy outcome-based diagnostic criteria and the frequent changes in the recommendations of many associations and organizations of international repute. Unfortunately, the changes proposed by many professional organizations are not backed by strong research data. The withdrawal of FPG-based GDM diagnosis before 24 GW by IADPSG was based on reports that early GDM diagnosis by an abnormal FPG value was poorly predictive of later GDM at 24-28 GW[17]. This approach has the limitation of considering pregnancy as a 'metabolically static state', having fixed glucose threshold values for all adverse events throughout pregnancy. In contrast, the HAPO study revealed a differential effect of the gestational age of onset of hyperglycemia on adverse events: PG values between 24-32 GW were associated with abnormalities in birth weight, while the HbA1c of that period (glycemia of preceding three months) led to preterm birth, primary cesarean delivery and preeclampsia[68]. Furthermore, several studies have suggested that hyperglycemia in early pregnancy *per se* can lead to significant adverse pregnancy events, even without the development of GDM in later pregnancy[63,66,67]. Hence, there is a strong need to identify glucose threshold values in early pregnancy, which can reliably predict adverse pregnancy events, and not GDM development alone, in later pregnancy. The differential effect of glycemia at different stages of pregnancy on adverse pregnancy events needs to be explored further. The mechanisms behind the deleterious effects of "mild hyperglycemia in early pregnancy" on fetal development and on adverse pregnancy events have not yet been clearly identified. Further research to identify any modifiable factors in early pregnancy will help to design preventive strategies for "hyperglycemia" in the peri-conception period and to develop alternate nonglucose centric measures.

There are significant ethnic and racial differences in PG and HbA1c threshold values for adverse pregnancy events, which was evident in two well-designed studies in Europe: Lower PG threshold values for LGA for South Asians than British Caucasians in the Bradford birth cohort by Farrar *et al*[29] and lower HbA1c (first trimester) threshold values for adverse events among South Central Asians compared to Caucasians in Spain by Mañé *et al*[69]. The ADA proposal of an HbA1c value of 41 mmol/mol in the first trimester to identify women prone to adverse events is derived from a New Zealand study involving predominantly Caucasian women[67]. The ADA-proposed HbA1c and FPG threshold values (≥ 6.1 mmol/L) for adverse pregnancy events were tested in a cohort of 2638 pregnant South Asian women in our center[4,39]. The percentage of women with adverse events identified by the ADA-proposed FPG and HbA1c threshold levels was significantly lower than the percentage of women having these events in the group of women with a diagnosis of IHEP by IADPSG criteria. Hence, an IHEP diagnosis identifies more South Asian pregnant women who are prone to adverse pregnancy events than those detected by the ADA-proposed FPG and HbA1c threshold values.

The trimester-related variations in the effect of hyperglycemia on fetal and adverse events and ethnic differences in the threshold for these adverse effects are major areas for future research. Despite having the highest number of women with HIP in the world, no center from South Asia (Indian subcontinent) was included in the HAPO study. There is a strong need for a HAPO-like study in early pregnancy among pregnant women of this region to identify the PG threshold values for various adverse events. Furthermore, the benefit of early intervention should be assessed in a randomized control trial. However, in obstetric practice, where early GDM screening and early initiation of GDM treatment have been common practices for several decades, withdrawing GDM treatment from women who have a diagnosis of "early GDM or IHEP" is a major challenge to researchers.

CONCLUSION

A significant number of pregnant women in South Asian countries have intermediate hyperglycemia in

early pregnancy. The current estimates suggest that one-third of GDM women among South Asian countries are diagnosed before the conventional screening period of 24-28 gestational weeks. The guidelines of regional professional bodies such as DIPSI and the local governmental guidelines strongly recommend screening for IHEP at the first prenatal visit. There is no consensus on the test or the criteria used for IHEP diagnosis in this region. Despite the controversies on the diagnostic threshold values, the OGTT is the preferred test for IHEP diagnosis in South Asia. Other tests, such as FPG and HbA1c, are routinely performed to detect DIP and hence can be considered potential tests for IHEP detection. The frequent changes in international guidelines on IHEP detection and management, without strong research data to justify these changes, have led to major confusion in obstetric practice in South Asian countries. The intervention studies among women with IHEP have yielded conflicting results, which is partly attributable to the heterogeneity in study design. However, there is some suggestion in these studies of a possible fetal effect of mild hyperglycemia in early pregnancy that may not be reversible with the normalization of blood glucose in later pregnancy. Further research to identify the exact pathogenetic mechanisms of maternal and fetal effects of IHEP is recommended.

FOOTNOTES

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MINIREVIEWS

Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes

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Abstract

Diabetes mellitus (DM) is still one of the most common diseases worldwide, and its prevalence is still increasing globally. According to the American and European recommendations, metformin is considered a first-line oral hypoglycemic drug for controlling type 2 DM (T2DM) patients. Metformin is the ninth most often prescribed drug in the world, and at least 120 million diabetic people are estimated to receive the drug. In the last 20 years, there has been increasing evidence of vitamin B12 deficiency among metformin-treated diabetic patients. Many studies have reported that vitamin B12 deficiency is related to the malabsorption of vitamin B12 among metformin-treated T2DM patients. Vitamin B12 deficiency may have a very bad complication for the T2DM patient. In this review, we will focus on the effect of metformin on the absorption of vitamin B12 and on its proposed mechanisms in hindering vitamin B12 absorption. In addition, the review will describe the clinical outcomes of vitamin B12 deficiency in metformin-treated T2DM.

Key Words: Metformin; Vitamin B 12 deficiency; Diabetes mellitus; Vitamin B12; Type 2 diabetes mellitus

Core Tip: In the last 20 years, there was increasing evidence of the presence of vitamin B12 deficiency among metformin-treated diabetic patients. Vitamin B12 deficiency may have a very bad complication for the T2DM patient. This review will focus on the effect of metformin on the absorption of vitamin B12 and on its proposed mechanisms in hindering vitamin B12 absorption. In addition to that, the review will describe the clinical outcomes of vitamin B12 deficiency in metformin-treated T2DM.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder diagnosed by abnormally high blood glucose levels. It is considered one of the most common diseases that lead to mortality and morbidity worldwide. Despite the development of health systems and public health concepts, the prevalence of DM is increasing globally[1]. According to current estimates, the number of people with diabetes in France and Belgium will rise by 17% by 2035, with an increase of 22% in the United States and the United Kingdom, 31% in Canada, and 3% to 37% in other European Union nations[2,3]. As known, uncontrolled DM may be the main cause of mortality among people[4]. The leading cause of morbidity and mortality in people with diabetes is vascular complications, which affect both the macrovascular system [cardiovascular disease (CVD)] and the microvascular system [diabetic kidney disease (DKD)], as well as diabetic retinopathy and neuropathy[5].

Metformin is considered one of the most important hypoglycemic drugs used to control the hyperglycemic state in patients with DM. It is mainly used in patients with Type 2 DM (T2DM) and both European and American recommendations recommend it as a first-line pharmacological treatment for T2DM[6-8]. According to many clinical trials, the drug improves cardiovascular outcomes in T2DM patients. Metformin is currently the most frequently given oral anti-diabetic drug because of its demonstrated efficacy, comparatively low risk, and potential for usage with other anti-diabetic drugs. More than 150 million diabetic patients are thought to receive the drug regularly worldwide[9].

In the last 20 years, there was increasing evidence of vitamin B12 deficiency among metformin-treated diabetic patients[10,11]. Many studies have reported that vitamin B12 deficiency is related to the malabsorption of vitamin B12 among metformin-treated T2DM patients[11-13]. Vitamin B12 deficiency may have terrible complications for T2DM patients, which should be considered during the therapeutic plan[12,14]. In this review, we will focus on the effect of metformin on the absorption of vitamin B12 and on its proposed mechanisms in hindering vitamin B12 absorption. In addition, the review will describe the clinical outcomes of vitamin B12 deficiency in metformin-treated T2DM and the impact of metformin use on serum vitamin B12.

OVERVIEW OF DIABETES

DM is a chronic metabolic disorder characterized to be an elevation in blood glucose levels caused by an absolute or relative insulin insufficiency, insulin resistance due to dysfunctional cells, or both. Other clinically discernible subtypes of diabetes exist, including monogenic diabetes (such as maturity-onset diabetes of the young or neonatal diabetes), gestational diabetes, and possibly a late-onset autoimmune form (latent autoimmune diabetes in adults)[12,15].

Diabetes is traditionally divided into an early-onset autoimmune form (T1D) and a late-onset non-autoimmune form (T2D). In fact, T2D is generally used to describe any type of diabetes that is not autoimmune or monogenic in origin, and it is becoming more widely acknowledged that it may reflect a collection of several pathophysiological states[4].

T1DM

Deficient insulin production is a hallmark of T1D, sometimes referred to as insulin-dependent, juvenile, or childhood-onset, which necessitates daily insulin therapy. T1D affected 9 million people in 2017, the majority of whom reside in high-income regions. Its etiology and prevention methods are unknown. Some of the symptoms are polyuria, polydipsia, polyphagia, weight loss, visual abnormalities, and exhaustion. These signs could appear out of nowhere[16].

T2DM

The body's inefficient use of insulin causes T2D; it is also known as non-insulin-dependent or adult-onset diabetes. T2D affects more than 95% of those who have the disease and is primarily caused by increased total body mass index and low physical activity[17].

Symptoms may be like those of T1D but are often less marked. As a result, the disease may be diagnosed several years after onset, after complications have already arisen. This type of diabetes was previously exclusively found in adults, but it is now increasingly common in kids as well.

Complications of DM

Both types of DM have a lot of complications in different vital systems in the human body. Diabetes is linked with long-term damage to both large and small blood vessels throughout the body, referred to as the macrovascular and microvascular systems[18]. Even though damage from high blood sugar to the macrovascular system, such as the coronary and cerebral arteries, is the primary cause of death in people with T2D, damage from high blood sugar to the microvascular system in the kidney, eyes, and nerves is far more frequent and significantly affects mortality[1].

Macrovascular complications: CVD is the primary cause of mortality for most of the diabetic population. Macrovascular problems are mostly caused by atherosclerotic constriction of arteries and veins, which results in cardiovascular, cerebrovascular, or peripheral artery diseases (PADs). Diabetes is a significant, manageable, independent risk factor for the development of CVD[19]. Cerebrovascular diseases, such as stroke and ischemia, occur in 20%-40% of diabetics due to atherosclerotic narrowing of the intracranial vessels and carotid artery. About 80% of diabetics over the age of 65 die of heart disease, and about 16% die from stroke[15,18].

Another very important complication is PAD which is an atherosclerotic occlusive disease of the lower extremities. And it carries a considerable risk of amputation for the affected limbs. One of the independent risk factors for the onset of PAD is DM. Particularly, diabetic people frequently experience critical limb ischemia, an advanced type of PAD that causes rest pain and long-term disability[20].

Microvascular complications: Uncontrolled hyperglycemic status can lead to microvascular complications like microangiopathy, nephropathy, neuropathy, and retinopathy by affecting small vessels, including capillaries[21]. One of the most common complications among diabetic patients is diabetes-related kidney dysfunction, also known as diabetic nephropathy or DKD. It is characterized by unusually high levels of albumin excretion with urine and a decreased glomerular filtration rate because of lesions that have developed in the glomerulus. In addition to that, people with long-term diabetes develop diabetic neuropathy because of chronic nerve damage[4,15,22].

Another common complication is diabetic retinopathy, where one-third of people with hypertension and high blood sugar are also diagnosed with diabetic retinopathy. Increased vascular permeability, the thickness of the retina, and neovascularization of the retina are its defining features, all of which cause vision loss. It is among the leading causes of long-term blindness and vision impairment in diabetics [23].

How can DM cause cell damage and consequently microvascular and macrovascular complications?

As we have mentioned before, the main feature of DM is the uncontrolled hyperglycemic state closely related to cell damage. Hyperglycemia causes an increase in reactive oxygen species (ROS) production, which causes oxidative stress in the body[18]. In normal circumstances, glucose is metabolized by way of the glycolysis pathways, followed by the tricarboxylic acid (TCA) cycle in mitochondria. This results in the generation of electron donors such as NADH (reduced nicotinamide adenine dinucleotide) and FADH2 (reduced flavin adenine dinucleotide), which play an essential role in transferring electrons to the molecular oxygen by the electron transport chain (ETC), so reducing the oxygen to water[18,24].

On the other hand, in the case of uncontrolled hyperglycemia, the increased rate of glucose oxidation in the TCA cycle increases the transport of electron donors into the ETC. This hinders the ETC, causing superoxide to be produced instead of water as the voltage gradient increases and reaches a critical threshold limit[18]. As a result, the increase in ROS-like superoxide in diabetic microvasculature can stimulate endothelial cell damage and, consequently a lot of micro and macrovascular complications.

OVERVIEW OF METFORMIN AND ITS ROLE IN THE TREATMENT OF DM

Metformin is a biguanide derivative and one of the most common oral anti-diabetic drugs. It is used mainly to treat T2D, especially in obese people. Compared to insulin, glibenclamide, and chlorpropamide, metformin has been found to reduce diabetes mortality and complications by 30%[7,11,25].

Metformin lowers serum glucose levels through several mechanisms without increasing insulin secretion. It is recognized as an insulin sensitizer because it enhances the cells' response to insulin[25]. In addition, metformin suppresses the liver's endogenous glucose synthesis primarily due to a decrease in the rate of gluconeogenesis and a minor impact on glycogenolysis. Furthermore, metformin stimulates insulin signaling and glucose transport in muscles while inhibiting critical enzymes involved in gluconeogenesis and glycogen production in the liver when the enzyme adenosine monophosphate kinase (AMPK) is activated[7,25,26].

Recent studies showed that metformin could reduce microvascular and macrovascular complications by its role in inhibiting the cell damage process in big and small vessels. This effect of metformin is mainly mediated by its action on AMP-activated kinase in tissues and its ability to reduce intracellular ROS[8,27]. In the same context, many studies showed that metformin could decrease the prevalence of nephropathy among diabetic patients by controlling oxidative stress and reversing the biochemical changes in renal tubules, consequently preventing tubular injury[28]. According to the previous

findings, metformin is currently the most frequently given oral anti-diabetic drug because of its demonstrated efficacy, comparatively low risk, and potential for usage with other anti-diabetic medications. 150 million diabetic individuals are estimated to receive the drug regularly worldwide[25].

Possible side effects of metformin

Metformin does not have many side effects, but it can result in lactic acidosis, a severe condition with the following symptoms: Dizziness, significant drowsiness, pain in the muscles, fatigue, chills, blue or pale skin, rapid or difficult breathing, slow or irregular heartbeat, stomach pain with diarrhea, nausea, or vomiting[29].

The possibility of lactic acidosis can increase in the presence of other conditions that cause a low level of oxygen in the blood or poor circulation (such as a recent stroke, congestive heart failure, or recent heart attack), heavy alcohol use, and dehydration[30]. While lactic acidosis is uncommon, gastrointestinal intolerance is one of the most frequently occurring side effects among metformin-treated T2DM patients[7].

Vitamin B12 malabsorption is another reported side effect of metformin usage in T2DM patients. There are varying degrees of evidence to support the link between metformin use and low vitamin B12 levels[13,31,32]. However, a few issues with the topic need to be clarified. Through this review, we will try to focus mainly on the possible relationship between metformin use and vitamin B12 deficiency.

METFORMIN AND VITAMIN B12 DEFICIENCY

In the last two decades, there has been an increasing interest in the relationship between metformin and vitamin B12 deficiency. The first report of metformin-associated vitamin B12 malabsorption was made in 1971 by Tomkin *et al*[33]. After that, many experimental, observational studies, and systematic reviews described the relationship between metformin and vitamin B12 deficiency in T2DM patients[6,13,31,32]. The effect of metformin on vitamin B12 absorption is also reported in metformin-treated polycystic ovary syndrome (PCOS) patients. A meta-analysis of six randomized controlled trials showed that metformin use caused dose-dependent drops in vitamin B12 levels in patients with T2DM or PCOS [34]. The importance of an accurate description of the association between the use of metformin and vitamin B12 comes from the significance of the clinical manifestations of vitamin B12 deficiency and its impact on the quality of diabetic patients' life. To better understand the relationship between metformin and vitamin B12 deficiency, we should have a good understanding of the nature of vitamin B12, the mechanism of its absorption, and how metformin can decrease its absorption.

Vitamin B12

Cobalamin, often known as vitamin B12, is a water-soluble vitamin that contains cobalt and functions as a co-factor for enzymes that are important for metabolism[35]. All cobalamin active in humans, such as cyanocobalamin, hydroxocobalamin, methyl cobalamin, and 5-deoxyadenosine cobalamin, are referred to as vitamin B12 (adenosyl-Cbl). However, different dosage forms of the first three types are offered as commercial products. The physiologically active forms of vitamin B12, adenosyl-Cbl, and methyl cobalamin, are produced intracellularly from all forms of the vitamin[5,36,37]. Vitamin B12 is an essential co-factor in intracellular enzyme activities involved in DNA synthesis and amino acid and fatty acid metabolism. In addition, it is necessary for erythropoiesis and the proper function of the central nervous system[35,37].

Absorption of vitamin B12

Vitamin B12 is absorbed by target cells through a difficult course that includes various proteins and receptors. Understanding the multistep process of vitamin B12 absorption is very important to understand the link between malabsorption of vitamin B12 in the presence of other medications like metformin.

Dietary vitamin B12 is generally founded in a protein-bound form. Protein-bound vitamin B12 is detached in the stomach because of gastric acid and pepsin. After that, the free vitamin is joined to R-binder, a salivary and gastric glycoprotein that shields vitamin B12 from the highly acidic stomach environment. R-binder is broken down by pancreatic proteases in the duodenum, releasing vitamin B12. The intrinsic factor (IF), a glycosylated protein released by stomach parietal cells, binds the free vitamin to create the IF vitamin B12 complex[5,38].

The IF-vitamin B12 complex passes *via* receptor-mediated endocytosis in the terminal ileum while avoiding proteolysis and acting as a carrier for the vitamin. The IF-vitamin B12 complex binds to the ileal cubilin receptor, a glycosylated protein expressed on the apical side of ileal enterocytes. Specific cubilin domains are engaged by the IF-vitamin B12 complex. And calcium cations are necessary for this interaction, where calcium can increase the complex's functional affinity to the receptor[39].

The ileal enterocyte then endocytoses the IF-vitamin B12-cubilin receptor complex. The IF-vitamin B12 complex separates from cubilin after endocytosis. When the complex enters the lysosome, IF is broken down and vitamin B12 crosses the membrane into the cytoplasm[39]. The vitamin then circulates

with transcobalamin-I (TC-I) or TC-II linked to it. 20%-30% of the total amount of circulating vitamin B12 is thought to be bound to the TC-II protein. Newly absorbed vitamins are bound by the protein and transported to the target tissues where they are absorbed *via* a receptor-mediated internalization process [5,39].

How can metformin cause malabsorption of vitamin B12?

Metformin can reduce the absorption of vitamin B12 through a mechanism that has not been established clearly[40]. Until now, several theories describe how metformin prevents the absorption of vitamin B12. These include compromised enterohepatic B12 circulation, increased vitamin B12 hepatic storage, decreased IF production, and decreased intestinal motility with bacterial overgrowth[12,40]. The most accepted theory is that metformin antagonizes the calcium cation and prevents the calcium-dependent IF-vitamin B12 complex from binding to the ileal cubilin receptor and consequently will reduce the endocytosis process of vitamin B12[5,12].

It is proposed that metformin could give a positive charge to the membrane's surface of cubilin receptor[41]. The positively charged receptor will push the divalent calcium cations by repulsion forces. This will lead to vitamin B12 malabsorption because the calcium-dependent binding of the IF-vitamin B12 complex to the ileal cubilin receptor is compromised[12]. Figure 1 shows how metformin can affect the absorption of vitamin B12.

CLINICAL OUTCOMES OF VITAMIN B12 DEFICIENCY IN METFORMIN-TREATED T2DM PATIENTS

As we mentioned before, many observational and experimental described the association between long-term metformin use and low vitamin B12 levels. The deficiency of vitamin B12 may lead to many clinical symptoms that may impact the quality of diabetic patients' life. In this review we tried to summarize the most important complications of vitamin B12 deficiency in metformin-treated T2DM patients through the following.

Neuropathy

Neuropathy is a primary complication of T2DM and a direct manifestation of vitamin B12 deficiency. Weakness, numbness, and pain are common symptoms of peripheral neuropathy, which develop when the peripheral nerves outside the brain and spinal cord are damaged. Many recent studies found that the long-term use of metformin could be a cause for increasing the prevalence of peripheral neuropathy among T2DM patients[13,31,42,43]. A recently published study showed a positive correlation between the period of metformin therapy and the severity of peripheral neuropathy[13].

The complications were not limited to peripheral neuropathy but also included autonomic cardiac neuropathy. In this context, Hansen *et al*[44] conducted a randomized, placebo-controlled trial that included 469 diabetic individuals who were using insulin and had an average duration of diabetes of 10 years. In this study, three cardiovascular reflex tests were used to evaluate the patients for cardiovascular autonomic neuropathy; after that, they were randomly assigned to either metformin or a placebo. The researchers observed that the vitamin B12 levels were steady with placebo after 18 months but dropped with metformin treatment. In addition, a significant reduction in orthostatic blood pressure in the metformin group indicated a worsening of cardiovascular autonomic neuropathy[44]. A recently published study reported that cardiac autonomic neuropathy is linked to cardiac events, cardiac arrhythmias, and sudden death. The study reported that cardiac autonomic neuropathy had been observed to be associated with a 3.16-fold [95% confidence interval (CI): 2.42-4.13, $P = 0.0001$] increase in cardiovascular disorders and a 3.17-fold increase (95%CI: 2.11-4.78, $P = 0.0001$) in mortality[45].

Neuropsychiatric disorders

The decrease in the absorption of vitamin B12 due to metformin may affect the treated patients' cognitive function since several studies connected the decline in cognitive functions and some depressive symptoms to low vitamin B12 levels[35]. A cohort study conducted by Porter *et al*[46] showed that metformin use was associated with decreased vitamin B12 and vitamin B6 levels along with an increased risk of cognitive dysfunction. Another two recent studies reported that metformin-treated patients suffering from vitamin B12 deficiency have lower cognitive function and a higher chance of developing depression[47,48].

Anemia

As metformin can cause vitamin B12 deficiency, it may cause anemia. Vitamin B12 deficiency can cause a delay in the maturation of red blood cells (RBCs) and many changes in their shape, leading to megaloblastic anemia. Megaloblastic anemias are characterized by an imbalance between nuclear and cytoplasmic maturation and abnormal nuclear maturation in RBCs. Vitamin B12 deficiency and a lack of folates affect DNA synthesis, which slows nuclear replication and postpones all stages of development

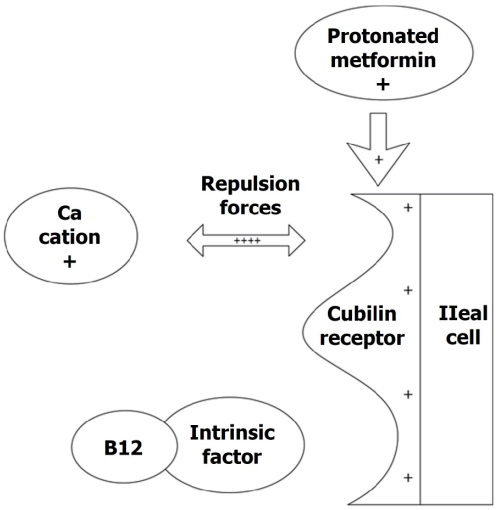

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Figure 1 Schematic diagram of how metformin can affect the absorption of vitamin B12.

[49,50].

Although many studies support the positive correlation between metformin use and vitamin B12 deficiency, there is still uncertainty about whether metformin causes anemia and whether this is triggered by B12 deficiency or not in metformin-treated T2DM patients[51,52]. In this context, Donnelly *et al*[53] made various statistical analyses using data exported from two randomized clinical trials and one observational study. The findings of this study showed that metformin use could cause a decrease in hemoglobin levels, and it is correlated to the early risk of anemia in individuals with T2DM. Unfortunately, the other previously performed high-quality evidence clinical studies on low vitamin B12 levels related to metformin did not investigate the significance of metformin use on hematological values. However, many case report studies linked megaloblastic anemia to the long-term use of metformin in T2DM patients[14,40,54].

Treatment of metformin-induced vitamin B12 deficiency

As we stated before, several studies including interventional studies, observational studies, and meta-analyses concluded that chronic use of metformin could be a cause of vitamin B12 deficiency, and many complications may accompany this. To avoid all these complications, vitamin B12 supplementation may be required[55]. In this context, a newly published systematic review including seven clinical trials showed that the application of vitamin B12 supplementation for metformin-treated T2DM patients will be valuable in preventing or treating vitamin B12 deficiency and neuropathy and should be considered during the T2DM management plan[32].

Similarly, a recently performed randomized, double-blind, placebo-controlled trial concluded that the treatment of metformin-treated patients with diabetic neuropathy with 1 mg of oral methylcobalamin for twelve months improved plasma B12 levels and improved all neurophysiological symptoms[56]. Since the typical amount of vitamin B12 stored in the liver is 2500 pg, it is believed that, in most cases, it will take at least five years of metformin use to deplete these reserves. However, other causes could increase the decrease of hepatic reserves, especially in the elderly due to the high prevalence of atrophic gastritis and proton pump inhibitor users. A recently published study reported that the monitoring of B12 levels might be important only for patients on long-term therapy of metformin (more than four years), especially when combined with proton pump inhibitors[57].

CONCLUSION

Metformin can cause vitamin B12 deficiency by reducing the absorption of the IF complex through the enteral cubilin receptor in the terminal ileum, which can either cause peripheral neuropathy, cardiac autonomic neuropathy, neuropsychiatric symptoms, or hematological disorders. The most severe side effect of metformin-induced vitamin B12 deficiency may be the development or acceleration of cardiac autonomic neuropathy, which is linked to an increase in cardiac arrhythmias, cardiac events, and mortality. Therefore, it is advised that people taking metformin undergo annual testing for vitamin B12 deficiency. In case of vitamin B12 deficiency, early replacement with intramuscular vitamin B12 to restore hepatic storage of vitamin B12 is recommended.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Association of bone turnover biomarkers with severe intracranial and extracranial artery stenosis in type 2 diabetes mellitus patients

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Abstract

BACKGROUND

Intracranial and extracranial artery stenosis is associated with cerebral infarction. Vascular calcification and atherosclerosis are the main causes of stenosis and major risk factors for cardiovascular and cerebrovascular events in patients with type 2 diabetes mellitus (T2DM). Bone turnover biomarkers (BTMs) are associated with vascular calcification, atherosclerosis, glucose, and lipid metabolism.

AIM

To investigate the association of circulating BTM levels with severe intracranial and extracranial artery stenosis in patients with T2DM.

METHODS

For this cross-sectional study including 257 T2DM patients, levels of the BTMs serum osteocalcin (OC), C-terminal cross-linked telopeptide of type I collagen (CTX), and procollagen type I N-peptide were measured by electrical chemiluminescent immunoassay, and artery stenosis was assessed by color Doppler and transcranial Doppler. Patients were grouped according to the existence and location (intracranial *vs.* extracranial) of artery stenosis. Correlations between BTM levels, previous stroke, stenosis location, and glucose and lipid metabolism were analyzed.

RESULTS

T2DM patients with severe artery stenosis had a higher frequency of previous stroke and levels of all three tested BTMs (all $P < 0.05$) than patients without. Some differences in OC and CTX levels were observed according to the location of artery stenosis. Significant associations were also observed between BTM levels and some glucose and lipid homeostasis parameters. On multivariate logistic regression analysis, all BTMs were significant predictors of artery stenosis in T2DM patients with and without adjustment for confounding factors (all $P < 0.001$), and receiver operating characteristic curve analysis demonstrated the

ability of BTM levels to predict artery stenosis in T2DM patients.

CONCLUSION

BTM levels were found to be independent risk factors for severe intracranial and extracranial artery stenosis and were differentially associated with glucose and lipid metabolism in patients with T2DM. Therefore, BTMs may be promising biomarkers and potential therapeutic targets for artery stenosis.

Key Words: Bone turnover biomarkers; Type 2 diabetes mellitus; Osteocalcin; C-terminal cross-linked telopeptide of type I collagen; Procollagen type I N-peptide; Intracranial and extracranial artery stenosis

Core Tip: The occurrence of cerebral infarction is associated with severe intracranial and extracranial artery stenosis; thus, this study aims to identify risk factors for severe intracranial and extracranial artery stenosis in patients with type 2 diabetes mellitus (T2DM) to prevent the occurrence of cerebral infarction. Our study found that Bone turnover biomarkers (BTMs) are associated with the risk of arterial stenosis, probably due to the relationship between BTMs and glucose and lipid metabolism disorders. Detection of BTMs in patients with T2DM may help reduce the occurrence of cardiovascular disease.

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INTRODUCTION

Intracranial and extracranial artery stenosis is an established predictor of cerebral infarction[1]. Therefore, a clear understanding of risk factors for intracranial and extracranial artery stenosis is important for the prevention of cerebrovascular events. Atherosclerosis is a critical stage of arterial stenosis, and abnormal bone metabolism is associated with the development of atherosclerosis[2]. Vascular calcification is one of the pathologic mechanisms of atherogenesis, and it is a programmed form of osteogenesis that is induced by inflammatory cytokines in blood vessels. The pathological process involves the trans-differentiation of vascular smooth muscle cells into osteoblasts, which are then associated with the activation of vascular osteogenesis, enhanced bone turnover, and abnormal mineral metabolism.

Atherosclerotic calcification is an independent predictor of the mortality and morbidity of cardiovascular and cerebrovascular diseases[3]. The extent and severity of vascular calcification are a reflection of the atherosclerotic plaque burden, and as an outcome of atherosclerosis, vascular calcification causes vascular sclerosis and dysfunction and is accepted as the basic pathological process in many cardiovascular diseases[4]. Vascular calcification is an active and regulated process with similarities to bone formation and is mediated by many of the same processes that promote bone formation[5]. Calcification of atherosclerotic plaques is considered a complex physiological process mediated by both inhibitor and promoter interactions, including between osteoclast- and osteoblast-like arterial cells. Plaque calcification is an important factor in plaque instability, which is conducive to plaque rupture and thrombosis. Accordingly, it is a predictor of future cardiovascular events.

Research shows that patients with type 2 diabetes mellitus (T2DM) who experience a transient ischemic attack or an anterior circulation ischemic stroke are more likely to have a lipid-rich necrotic core and vascular plaques with calcification, and the biological processes of bone formation and calcified atherosclerotic plaque share many common features in patients with T2DM[6]. Additionally, vascular calcification is considered a major risk factor for T2DM and is always associated with cardiovascular complications.

Bone turnover biomarkers (BTMs) are indicators of bone metabolism and may be related to the progression of vascular calcification. Bone metabolism is associated with dyslipidemia and diabetes, which are both important risk factors for atherosclerosis. BTM levels are also related to glucose and lipid metabolism[7]. Thus, BTM levels may be useful biomarkers for assessing the risk of cardiovascular events in patients with T2DM.

Osteocalcin (OC) is a biochemical marker of bone formation that specifically mediates bone mineralization and is expressed mainly by osteoblasts. Osteoblasts are also known to secrete endocrine factors that regulate insulin production and adipose tissue metabolism. OC can extend the metabolic endocrine function of bone with obvious extraosseous effects and is vital for not only bone metabolism but also lipid and glucose metabolism. OC can directly regulate energy metabolism, including glucose and lipids, and is involved in the process of vascular calcification affecting the progression of atherosclerosis [8]. OC is also associated with atherosclerotic disease specifically in patients with T2DM[9]. Another BTM, the C-terminal cross-linked telopeptide of type I collagen (CTX), is a marker of bone resorption. Recent clinical research identified CTX as an independent predictor of increased common carotid artery wall intima - media thickness in the elderly population[10]. The BTM procollagen type I N-peptide (PINP) was shown to predict the risk of myocardial infarction in older male patients[11].

Clinical studies to date have mainly studied the associations between different BTMs and atherosclerosis, whereas few studies have explored the correlations between BTM levels and severe intracranial and extracranial artery stenosis in patients with T2DM. Thus, we aimed to determine whether BTMs are associated with intracranial and extracranial atherosclerosis and investigate the value of BTMs as potential indicators for risk assessment and intervention targets for severe intracranial and extracranial artery stenosis in patients with T2DM.

MATERIALS AND METHODS

Study sample and data collection

This cross-sectional study included 257 consecutive patients with T2DM who were hospitalized in our facility between January 2018 and December 2019 and underwent evaluation of intracranial and extracranial arteries. Data were recorded by trained physicians who used a standardized questionnaire to collect medical history information, including age, sex, and history of stroke, coronary artery disease (CAD), hypertension, dyslipidemia, smoking, and alcohol consumption. Diseases were recorded based on codes from the International Classification, Ninth Revision. Physical parameters, including height, weight, and body mass index (BMI), were measured. T2DM was diagnosed according to criteria recommended by the American Diabetes Association in 1997[12]. T2DM diagnosis was based on fasting plasma glucose ≥ 7.0 mmol/L or plasma glucose ≥ 11.1 mmol/L or hemoglobin (HbA1c) ≥ 6.5%. Patients were excluded if they were taking any medication that might influence bone metabolism, such as steroids, thiazides, vitamin D or calcium supplements, antiresorptive or hormone therapy, or were known to have any of the following conditions: acute diabetic complications, malignant tumor, infection, hepatic failure, chronic kidney disease, heart failure, thyroid disease, parathyroid disease, or gastrointestinal disease. The ethics committee of Xuanwu Hospital of Capital Medical University approved this study. All participants provided informed consent.

Biochemical measurements

After overnight fasting for 12 h, peripheral venous blood samples were collected early the next morning for all patients. Fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum creatinine (Scr), blood urea nitrogen (Bun), and uric acid (UA) levels were measured by an automatic analyzer. The glycated HbA1c level was measured by high-pressure liquid chromatography. Insulin (INS), C-peptide (CP), OC, CTX, and PINP levels were determined using an electrical chemiluminescent immunoassay.

Ultrasonography

Sonographers performed color Doppler ultrasound to obtain images of the common carotid arteries, external carotid arteries, internal carotid arteries, and subclavian arteries. Severe extracranial stenosis was defined as the narrowing of vessel diameter by ≥ 70% in any of these arteries. Sonographers also performed transcranial Doppler ultrasound to obtain images of the anterior cerebral arteries, middle cerebral arteries, posterior cerebral arteries, basilar arteries, and vertebral arteries. Severe intracranial artery stenosis was defined as the narrowing of vessel diameter by ≥ 70% in any of these arteries. According to the results of these examinations, patients were assigned to four groups: No severe intracranial and extracranial artery stenosis (NOCS) group, only severe intracranial artery stenosis (ICAS) group, only severe extracranial artery stenosis (ECAS) group, and combined severe intracranial and extracranial artery stenosis (COAS) group. For analysis, the AS group included all patients with severe intracranial and/or extracranial artery stenosis (ICAS + ECAS + COAS groups).

Statistical analysis

Normally distributed data are presented as the mean ± SD, and data with a skewed distribution are presented as the median (interquartile range). Categorical variables are shown as frequencies (percentages). We used the Mann–Whitney *U* test to compare mean values between the NOCS and AS groups. The Kruskal–Wallis test was used to compare mean values among the NOCS, ICAS, ECAS, and

COAS groups. When no subgroup had an expected count below five, we used the Pearson χ^2 test. If any subgroup had an expected count below five, we used Fisher's exact test. We performed Spearman correlation analysis to calculate correlation coefficients between BTM levels and glucose and lipid parameters. We used multivariate logistic regression models to estimate the association of artery stenosis with BTM levels. Receiver operating characteristic (ROC) curves and the corresponding area under the curve (AUC) values were used to examine the ability of BTM levels to predict the incidence of artery stenosis. All statistical analyses were performed with the SPSS Statistical Package (version 26.0). The results were considered statistically significant if the corresponding *P* value was less than 0.05.

RESULTS

Patient characteristics

Among the 257 T2DM patients included in this study, 136 were female and 121 were male. The mean age of all participants was 66 ± 11 years (range, 36–93 years). The average duration of T2DM among all patients was 12.0 ± 13.0 years. Overall, 87.2% (n = 224) had no stenosis (NOCS group), and 12.8% (n = 33) had some form of stenosis (AS group). The clinical and laboratory parameters for all patients and the groups with and without artery stenosis are presented in Table 1. Compared with the NOCS group, the AS group had a higher percentage of patients with a history of stroke (9.8% *vs* 27.3%, P = 0.004) and higher levels of HbA1c, OC, CTX, and PINP (all P < 0.05).

The clinical and laboratory parameters of patients categorized further based on the location of artery stenosis are presented in Table 2. Among the 33 patients with T2DM with artery stenosis, 7.4% (n = 19) had only severe intracranial artery stenosis (ICAS group), 3.1% (n = 8) had only severe extracranial artery stenosis (ECAS group), and 2.3% (n = 6) had both severe intracranial and extracranial artery stenosis (COAS group). Compared with that in the NOCS group, the frequency of previous strokes was higher in all three groups (21.1% in ICAS group *vs* 25.0% in ECAS group *vs* 50.0% in COAS group *vs* 9.8% in NOCS, P = 0.009). Compared with the NOCS group, the ICAS group had a lower frequency of hyperlipidemia, and the ECAS group had a higher frequency of hyperlipidemia (21.1% in the ICAS group *vs* 75.0% in the ECAS group *vs* 50% in the NOCS group, P = 0.029). The OC levels differed significantly among the four groups (P = 0.012). Further statistical analyses determined that the OC levels in the COAS and ICAS groups were higher than those in the NOCS group (P = 0.034 and 0.039, respectively). While the ECAS group showed a trend toward higher OC levels compared with the NOCS group, the difference was not significant (P = 0.077). Similarly, the CTX levels differed among the four groups (P = 0.027), and further statistical analysis showed that the CTX level in the COAS group was higher than that in the NOCS group (P = 0.017). The ICAS group also showed a trend toward higher CTX levels than the NOCS group, but this difference did not reach statistical significance (P = 0.062). The PINP concentrations were comparable among the four groups, although a weak trend toward a reduced PINP was seen in the NOCS group (P = 0.059).

Associations between BTM levels and artery stenosis

We applied four multivariate logistic regression models to identify independent predictors of artery stenosis (Table 3). With the unadjusted Model 1, the OC level was found to be significantly associated with an increased risk of artery stenosis [odds ratio (OR) = 1.123; 95%CI: 1.049–1.203; P = 0.001]. A similar association was observed with the model further adjusted for age and sex (Model 2, OR = 1.117; 95%CI: 1.041–1.199; P = 0.002). With further adjustment of the model for hypertension, hyperlipidemia, CAD, duration of T2DM, smoking status, and drinking status, the OC level remained a predictor of high risk for artery stenosis (Model 3, OR = 1.117; 95%CI: 1.039–1.201; P = 0.003). A significant association between the OC level and artery stenosis persisted after further adjustment of the model for BMI, Scr, Bun, UA, and HbA1c (Model 4, OR = 1.109; 95%CI: 1.029–1.196; P = 0.007). CTX and PINP levels were also significantly associated with artery stenosis in all models with adjustment for different potential confounding factors (all P < 0.05).

Correlation of BTM levels and parameters of glucose and lipid metabolism

The results of the Spearman correlation analysis of associations between BTM levels and parameters of glucose and lipid metabolism are presented in Table 4. This analysis showed that the OC level was associated with the levels of TC (r = 0.185; P = 0.003) and LDL-C (r = 0.213; P = 0.001). Additionally, the CTX level was associated with the levels of FPG (r = –0.134; P = 0.031), TC (r = 0.147; P = 0.018), and LDL-C (r = 0.197; P = 0.003). The PINP level was associated with the levels of FPG (r = –0.141; P = 0.024) and LDL-C (r = 0.129; P = 0.039).

Predictive ability of BTM levels for artery stenosis

The ROC curves for the abilities of BTM levels to predict the incidence of artery stenosis in patients with T2DM are shown in Figure 1. The AUC (95%CI) values were 0.673 (0.580, 0.766) for OC, 0.644 (0.538, 0.751) for CTX, and 0.639 (0.536, 0.742) for PINP. All three BTMs were found to be significant predictors

Table 1 Basic and clinical characteristics of all participants and those in the no severe intracranial and extracranial artery stenosis and artery stenosis groups

Characteristics	All patients (*n* = 257)	NOCS group (n = 224)	AS group (*n* = 33)	*P* value
Age (yr)	66.0 (11.0)	66.0 (11.0)	68.0 (12.0)	0.236
Male, *n* (%)	121 (47.1)	103 (46.0)	18 (54.5)	0.358
Duration of T2DM (yr)	12.0 (13.0)	12.0 (14.0)	13.0 (11.0)	0.438
Risk factors, *n* (%)				
Hypertension	189 (73.5)	165 (73.7)	24 (72.7)	0.910
Hyperlipidemia	124 (48.2)	112 (50.0)	12 (36.4)	0.143
CAD	74 (28.8)	63 (28.3)	11 (33.3)	0.548
Previous stroke	31 (12.1)	22 (9.8)	9 (27.3)	0.004
Current smoker	74 (28.8)	67 (29.9)	7 (21.2)	0.303
Current drinker	64 (24.9)	59 (26.3)	5 (15.2)	0.165
BMI (kg/m^2)	25.76 (3.57)	25.78 (3.59)	25.76 (3.45)	0.597
Glucose (mmol/L)	7.48 (3.58)	7.49 (3.53)	6.72 (4.25)	0.563
HbA1c (%)	7.60 (2.50)	7.60 (2.60)	8.40 (2.90)	0.037
INS (μIU/mL)	12.21 (12.71)	12.00 (10.71)	16.82 (18.93)	0.159
CP (ng/mL)	2.33 (1.52)	2.33 (1.53)	2.40 (1.89)	0.754
TC (mmol/L)	4.26 (1.50)	4.27 (1.41)	4.23 (2.25)	0.721
TG (mmol/L)	1.48 (1.38)	1.50 (1.39)	1.41 (1.36)	0.433
LDL-C (mmol/L)	2.38 (1.27)	2.39 (1.20)	2.27 (1.79)	0.781
HDL-C (mmol/L)	1.12 (0.47)	1.13 (0.47)	1.04 (0.38)	0.320
Scr (μmol/L)	62.0 (24.0)	62.0 (22.8)	60.0 (43.0)	0.144
Bun (mmol/L)	5.98 (2.30)	5.92 (2.12)	6.82 (3.60)	0.137
UA (μmol/L)	341.0 (126.5)	342.0 (127.3)	339.0 (1520)	0.823
OC (ng/mL)	10.60 (5.87)	10.27 (5.87)	12.63 (7.07)	0.001
CTX (pg/mL)	0.31 (0.21)	0.29 (0.19)	0.39 (0.30)	0.007
PINP (pg/mL)	34.40 (20.96)	33.39 (20.37)	43.92 (25.50)	0.010

Data are presented as the mean ± SD unless otherwise indicated. NOCS: No severe intracranial and extracranial artery stenosis; AS: Artery stenosis; CAD: Coronary artery disease; BMI: Body mass index; HbA1c: Hemoglobin; INS: Insulin; CP: C-peptide; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; Scr: Serum creatinine; Bun: Blood urea nitrogen; UA: Uric acid; OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

of artery stenosis risk among the patients with T2DM included in this study ($P < 0.001$).

DISCUSSION

In this study, we explored the associations among BTM levels, previous stroke, and the burden and location of intracranial and extracranial artery stenosis in patients with T2DM. We also investigated the correlations among BTM levels and parameters of glucose and lipid metabolism in patients with T2DM. From the cross-sectional data analyzed in this study, we observed a considerable incidence of artery stenosis among patients with T2DM. Moreover, T2DM patients with artery stenosis were more likely to have a history of stroke than those without artery stenosis, regardless of the location of artery stenosis, and the highest incidence of previous stroke was observed among patients with both severe intracranial and extracranial artery stenosis (COAS group). We also found that BTM levels showed significant correlations with the risk of severe intracranial and extracranial artery stenosis. In this study, circulating OC, CTX, and PINP concentrations were significantly higher in T2DM patients with artery stenosis (AS group) than in those without artery stenosis (NOCS group, Table 1). Some differences were observed

Table 2 Basic and clinical characteristics of patients in the no severe intracranial and extracranial artery stenosis, intracranial artery stenosis, severe extracranial artery stenosis and combined severe intracranial and extracranial artery stenosis groups

Characteristics	NOCS group (*n* = 224)	ICAS group (*n* = 19)	ECAS group (*n* = 8)	COAS group (*n* = 6)	*P* value
Age (yr)	66.0 (11.0)	65.0 (16.0)	67.5 (13.0)	71.0 (10.0)	0.373
Male, *n* (%)	103 (46.0)	10 (52.6)	5 (62.5)	3 (50)	0.780
Duration of T2DM (yr)	12.0 (14.0)	15.0 (11.0)	18.0 (20.8)	11.5 (7.0)	0.635
Risk factors, *n* (%)					
Hypertension	165 (73.7)	13 (68.4)	5 (62.5)	6 (100)	0.527
Hyperlipidemia	112 (50.0)	4 (21.1)	7 (75.0)	2 (33.3)	0.029
CAD	63 (28.3)	7 (36.8)	2 (25.0)	2 (33.3)	0.826
Previous stroke	22 (9.8)	4 (21.1)	2 (25.0)	3 (50.0)	0.009
Current smoker	67 (29.9)	4 (21.1)	1 (12.5)	2 (33.3)	0.682
Current drinker	59 (26.3)	4 (21.1)	1 (12.5)	0 (0)	0.554
BMI (kg/m^2)	25.78 (3.59)	26.06 (3.90)	25.32 (2.33)	23.96 (12.48)	0.821
FPG (mmol/L)	7.49 (3.53)	7.83 (4.13)	7.13 (6.89)	5.76 (2.48)	0.446
HbA1c (%)	7.60 (2.60)	7.90 (3.10)	9.15 (1.70)	7.90 (1.80)	0.067
INS (μIU/mL)	12.00 (10.71)	16.99 (19.11)	12.78 (19.72)	15.90 (25.32)	0.334
CP (ng/mL)	2.33 (1.53)	2.52 (1.45)	1.56 (2.95)	2.03 (2.31)	0.599
TC (mmol/L)	4.27 (1.41)	3.85 (1.98)	4.92 (2.35)	4.35 (2.25)	0.535
TG (mmol/L)	1.50 (1.39)	1.59 (1.31)	1.29 (1.31)	1.16 (1.85)	0.585
LDL-C (mmol/L)	2.39 (1.20)	2.27 (1.76)	2.62 (1.88)	2.01 (2.27)	0.943
HDL-C (mmol/L)	1.13 (0.47)	0.98 (0.29)	1.25 (1.21)	1.04 (0.43)	0.170
Scr (μmol/L)	62.0 (22.8)	59.0 (48.0)	78.5 (44.8)	60.0 (40.3)	0.499
Bun (mmol/L)	5.92 (2.12)	6.36 (2.74)	7.92 (6.76)	6.57 (5.00)	0.419
UA (μmol/L)	342.0 (127.3)	358.0 (174.0)	322.5 (153.3)	303.0 (136.3)	0.746
OC (ng/mL)	10.27 (5.87)	12.76 (8.14)	11.89 (4.02)	15.15 (11.43)	0.012
CTX (pg/mL)	0.29 (0.19)	0.41 (0.29)	0.35 (0.23)	0.65 (0.51)	0.027
PINP (pg/mL)	33.39 (20.37)	41.25 (24.71)	47.22 (39.85)	51.28 (37.96)	0.059

Data are presented as the mean ± SD unless otherwise indicated. NOCS: No severe intracranial and extracranial artery stenosis; ICAS: Severe intracranial artery stenosis; ECAS: Severe extracranial artery stenosis; COAS: Combined severe intracranial and extracranial artery stenosis; CAD: Coronary artery disease; BMI: Body mass index; HbA1c: Hemoglobin; INS: Insulin; FPG: Fasting plasma glucose; CP: C-peptide; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; Scr: Serum creatinine; Bun: Blood urea nitrogen; UA: Uric acid; OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

among the trends in BTMs according to the location of artery stenosis. OC levels were higher in all subgroups with artery stenosis, whereas CTX levels were only higher in the COAS and ICAS groups than in the NOCS group. Our analyses indicated that all three BTMs were independent risk factors for severe intracranial and extracranial artery stenosis in patients with T2DM, in support of our hypothesis, and these associations were independent of possible confounders. Based on their correlation with indicators of glucose and lipid metabolism, the elevated BTM levels identified a particularly unfavorable metabolic profile, mostly related to dyslipidemia. Higher BTM levels were associated with elevated LDL-C, and the OC level was positively correlated with the TC level. However, the CTX level was negatively correlated with FPG and positively correlated with TC. Furthermore, the PINP level was negatively correlated with FPG. These findings suggest that BTM levels can reflect altered glucose and lipid metabolism as well as atherosclerosis. The role of BTMs in bone metabolism along with their influence on glucose and lipid homeostasis and stroke risk confirms the tight interaction of the cardiovascular–bone metabolism axis. Overall, the present study suggests that BTMs may represent novel biomarkers of accelerated atherosclerosis in patients with T2DM, offering promising tools for cardiovascular risk stratification among patients with T2DM.

Table 3 Association of bone turnover biomarker levels with artery stenosis

Variable	Model	OR (95%CI)	*P* value
OC	Model 1	1.123 (1.049-1.203)	0.001
	Model 2	1.117 (1.041-1.199)	0.002
	Model 3	1.117 (1.039-1.201)	0.003
	Model 4	1.109 (1.029-1.196)	0.007
CTX	Model 1	9.750 (1.759-54.059)	0.009
	Model 2	8.674 (1.529-49.211)	0.015
	Model 3	10.833 (1.725-68.037)	0.011
	Model 4	8.526 (1.189-61.119)	0.033
PINP	Model 1	1.024 (1.006-1.042)	0.010
	Model 2	1.022 (1.003-1.041)	0.025
	Model 3	1.022 (1.003-1.042)	0.021
	Model 4	1.020 (1.001-1.040)	0.044

Model 1: Unadjusted, crude model; Model 2: Adjusted for age and gender; Model 3: Further adjusted for hypertension, hyperlipidemia, coronary artery disease, duration of type 2 diabetes mellitus, current smoker, current drinker; Model 4: Further adjusted for body mass index, serum creatinine, blood urea nitrogen, uric acid, and hemoglobin. OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

Table 4 Spearman correlation coefficients for the associations between bone turnover biomarker levels and indicators of glucose and lipid metabolism

Variable	OC		CTX		PINP	
	r	*P* value	*r*	*P* value	*r*	*P* value
FPG	-0.102	0.103	-0.134	0.031	-0.141	0.024
HbA1c	-0.012	0.846	-0.022	0.724	-0.012	0.845
INS	-0.050	0.425	-0.086	0.172	-0.031	0.628
CP	0.021	0.741	0.045	0.473	0.067	0.286
TC	0.185	0.003	0.147	0.018	0.112	0.074
TG	0.020	0.753	-0.058	0.351	-0.041	0.514
LDL-C	0.213	0.001	0.187	0.003	0.129	0.039
HDL-C	0.020	0.745	0.062	0.319	0.015	0.811

OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide; FPG: Fasting plasma glucose; HbA1c: Hemoglobin; INS: Insulin; CP: C-peptide; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol.

T2DM is a common metabolic disease, and its incidence continues to increase worldwide. The incidence of atherosclerosis in patients with T2DM is high, and large vessel disease due to atherosclerosis seriously threatens the life and health of patients with T2DM. Thus, effective strategies for the prevention and treatment of large vessel disease in the context of T2DM are needed to improve public health globally. Even with reductions in traditional risk factors for cardiovascular disease (CVD), achieved *via* better blood lipid control and smoking cessation, the incidence of CVD remains high. Moreover, blood glucose control does not completely reduce the mortality attributed to atherosclerotic and vascular calcification-related CVD among patients with T2DM. Therefore, other pathological mechanisms of atherosclerosis need to be characterized to support the discovery of therapeutic targets that can be used to identify, diagnose, evaluate, and treat patients with T2DM at high risk of atherosclerosis as early as possible to improve the prognosis of patients with T2DM.

With research advances related to bone health and vascular lesions, the significance and key role of the bone–vascular axis in vascular lesions are increasingly recognized. The instability of atherosclerotic plaques is associated with a higher level of calcification, and severe vascular calcification can be

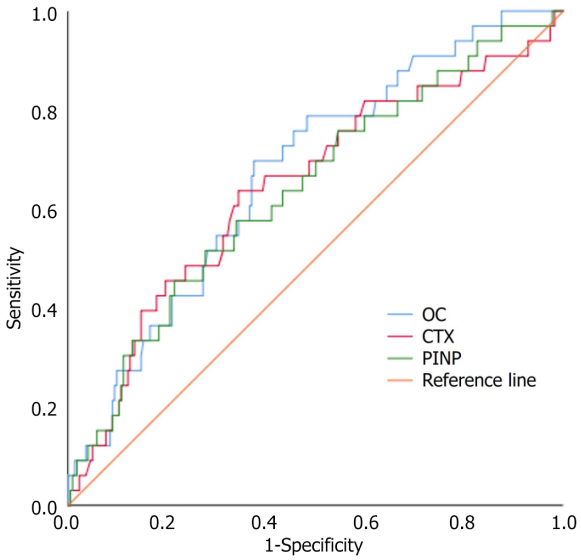

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Figure 1 Receiver operating characteristic curve analysis of the diagnostic value of bone turnover biomarker levels for artery stenosis in type 2 diabetes mellitus patients. OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

regarded as a nonspecific marker of atherosclerosis. The calcified atherosclerosis burden is considered the main predictor of risk for CVD events and death[13]. T2DM is associated with greater intraplaque calcification volume and a greater proportion of calcification within a plaque. Vascular calcification was also shown to be a powerful risk factor for cardiovascular death in patients with T2DM and results in the development of severe atherosclerosis[14]. Likely, several important mediators of bone mineral homeostasis are also involved in the development of arterial calcification.

Several mineralization markers have been identified in atheromatic plaques[15]. Currently, the effects of BTM levels on atherosclerosis or plaque calcification remain unclear, with conflicting data reported in the literature. Previous studies have mainly examined the relationship between BTM levels and the prevalence of atherosclerotic diseases and found that BTM levels may be predictors of cardiovascular risk in T2DM patients[16]. In patients with T2DM, OC was identified as an independent risk factor for carotid atherosclerosis[17] and shown to play a role in CVD[18]. OC was also found to be deposited at sites of vascular calcification[19]. Another study found that progressive calcification of atherosclerotic plaques was accompanied by a significant increase in OC[20]. Type I collagen is a major collagen component of the intima, media, and adventitia of blood vessel walls and is significantly increased in atherosclerosis. PINP is a precursor of type I collagen, and CTX is the carboxyl-terminal degradation product of type I collagen. Thus, the expression levels of both BTMs are increased in atherosclerosis accordingly. Abnormal matrix collagen turnover in vessel walls eventually causes arterial stiffness, which is associated with CVD risk, and increased arterial stiffness is associated with both collagen degradation (CTX) and synthesis[21]. Increased osteoclast activity has been observed in T2DM[22], and CTX expression was found in areas of intimal hyperplasia and late-calcified plaques[23]. Gafane *et al*[24] found a positive association between large artery stiffness and the CTX level. Another study found that OC and CTX levels are related to an increased risk of cardiac and carotid calcified plaque development [25]. However, research on the correlation between BTM levels and severe intracranial and extracranial stenosis in patients with T2DM is lacking. The present study found that BTM levels were significantly associated with the risk of artery stenosis in unadjusted and fully adjusted models accounting for potential confounders. These results suggest for the first time that BTMs are independent risk factors for severe intracranial and extracranial stenosis in patients with T2DM. Notably, these associations were independent of other known cardiovascular risk factors in T2DM. Additionally, our results indicate that BTMs may participate in and contribute to the pathogenesis and progression of atherosclerosis.

The mechanisms by which BTMs influence atherosclerosis remain unclear. One possibility is that OC plays an important role in glucose metabolism[26,27]. Consistently, in this study, OC was significantly associated with glucose regulation. We also observed that PINP and CTX levels were negatively correlated with FPG. A previous study found that PINP is negatively correlated with FPG and HbA1c [28]. We also found that all tested BTMs were associated with indicators of dyslipidemia. Barchetta *et al* [29] also reported an association between OC and TC. We also observed differences in glucose and lipid metabolism about artery stenosis in patients with T2DM. Patients with artery stenosis had higher levels of HbA1c than those without artery stenosis. However, indicators of dyslipidemia differed between patients with intracranial *vs* extracranial artery stenosis in our study. Jin *et al*[30] found that hyperlipidemia was an independent predictor of extracranial artery stenosis but not intracranial artery stenosis,

and in the present study, a higher frequency of hyperlipidemia was observed in the ECAS group.

Metabolic variables may represent the underlying mechanism for the association between BTMs and atherosclerosis, as increased BTM levels correlated with several metabolic risk factors and increased atherosclerosis. BTMs may influence glucose and lipid homeostasis, while cardiovascular risk factors can regulate the expression of mineralization markers and promote the calcification process.

The strengths of the present study include overall profiling of BTM levels in patients with T2DM, which has not been reported previously, and extensive correlation analyses between BTM levels and indicators of glucose metabolism, lipid metabolism, and vascular disease, which ultimately allowed us to identify independent associations between BTM levels and atherosclerosis in patients with T2DM. However, this study also has several limitations. Due to its cross-sectional design, we could not establish causal relationships among the correlating factors. Further prospective studies with relevant clinical endpoints are needed to clarify whether BTMs play a causal role in the development of atherosclerosis and to assess the effect of BTM-lowering therapies on the development of atherosclerosis. We plan to carry out large-scale longitudinal studies in the future. Finally, in vivo animal studies are required to elucidate the role of BTMs in the pathogenic mechanisms of atherosclerosis and plaque calcification.

Based on our findings that BTM levels are strongly correlated with artery stenosis risk and disturbance of glucose and lipid metabolism, BTMs should be investigated as indicators for predicting the risk of CVD and as potential therapeutic targets for artery stenosis in patients with T2DM. Multimodal imaging technologies can identify intracranial and extracranial atherosclerosis, which indicates an increased risk of ischemic events or artery stenosis. The combination of such imaging analyses and measurement of serum BTM levels offers a promising strategy for evaluating the diagnosis, pathogenesis, and progression of intracranial and extracranial stenosis. Therefore, we should test BTMs in patients with T2DM, complete vascular examinations in patients with elevated BTMs, and conduct active monitoring, follow-up, and treatment in this part of the population.

CONCLUSION

Elevated BTM levels correlated with an increased risk of artery stenosis in patients with T2DM as well as with several indicators of metabolic syndrome. Accordingly, BTM levels may serve as circulating endocrine markers that reflect the regulation of glucose and lipid metabolism, thereby reflecting the risk of vascular disease in patients with T2DM. BTMs may also represent potential therapeutic targets for atherosclerosis, and additional research is warranted to explore the underlying mechanism linking BTMs and atherosclerosis. Patients with high BTM levels should be considered a high-risk group in efforts to prevent cardiovascular events.

ARTICLE HIGHLIGHTS

Research background

Intracranial and extracranial artery stenosis is associated with cerebral infarction. Vascular calcification and atherosclerosis are the main causes of stenosis and major risk factors for cardiovascular and cerebrovascular events in patients with type 2 diabetes mellitus (T2DM).

Research motivation

Our study found that bone turnover biomarkers (BTMs) are associated with the risk of arterial stenosis, probably due to the relationship between BTMs and glucose and lipid metabolism disorders. Detection of BTMs in patients with T2DM may help reduce the occurrence of cardiovascular disease.

Research objectives

This study aimed to investigate the association of circulating BTM levels with severe intracranial and extracranial artery stenosis in patients with T2DM.

Research methods

After overnight fasting for 12 h, peripheral venous blood samples were collected early the next morning for all patients. Fasting plasma glucose, triglyceride, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, serum creatinine, blood urea nitrogen, and uric acid levels were measured by an automatic analyzer. The glycated hemoglobin (HbA1c) level was measured by high-pressure liquid chromatography. Insulin, C-peptide, osteocalcin (OC), C-terminal cross-linked telopeptide of type I collagen (CTX), and procollagen type I N-peptide (PINP) levels were determined using an electrical chemiluminescent immunoassay.

Research results

Among the 257 T2DM patients included in this study, 136 were female and 121 were male. The mean age of all participants was 66 ± 11 years. The average duration of T2DM among all patients was 12.0 ± 13.0 years. Overall, 87.2% had no stenosis, and 12.8% had some form of stenosis. Compared with the no severe intracranial and extracranial artery stenosis group, the artery stenosis group had a higher percentage of patients with a history of stroke and higher levels of HbA1c, OC, CTX, and PINP.

Research conclusions

Elevated BTM levels correlated with an increased risk of artery stenosis in patients with T2DM as well as with several indicators of metabolic syndrome. Accordingly, BTM levels may serve as circulating endocrine markers that reflect the regulation of glucose and lipid metabolism, thereby reflecting the risk of vascular disease in patients with T2DM.

Research perspectives

BTMs may also represent potential therapeutic targets for atherosclerosis, and additional research is warranted to explore the underlying mechanism linking BTMs and atherosclerosis.

FOOTNOTES

Author contributions: Si SC and Yang W contributed to the study conception and design; Liu J, Luo HY, Ma YX and Zhao H recruited patients and supervised the study process; Si SC analyzed the data and wrote the manuscript; and all authors read and approved the final article.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Efficacy of multigrain supplementation in type 2 diabetes mellitus: A pilot study protocol for a randomized intervention trial

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Abstract

BACKGROUND

Uncontrolled type 2 diabetes mellitus (T2DM) may lead to microvascular complications (nephropathy, retinopathy, and neuropathy) and cardiovascular diseases. The beta-glucan content in grains has the potential to improve insulin sensitivity, lowering postprandial glucose response and reducing inflammation degrees. A proper combination of grains not only satisfies human body's need, but also provides essential and reasonable nutritional contents. However, no trial has been conducted to evaluate the roles of multigrain in T2DM.

AIM

To determine the efficacy of multigrain supplementation among T2DM patients.

METHODS

From October 2020 to June 2021, a total of 50 adults living with T2DM, who were receiving standard diabetes care at Day Care Clinic, were randomized into either a supplementation group or a control group. The supplementation group received twice daily 30 g multigrain supplement (equivalent to 3.4 g beta-glucan) with standard medication for 12 wk, while the control group was prescribed with standard medication. Parameters such as glycemic control (HbA1c, FPG, and HOMO-IR), cardiometabolic profile (lipid profile, renal function test, and liver function test), oxidative stress status, nutritional status, and quality of life (QoL) were assessed at two time points: Baseline and the end of the treatment period (week 12).

RESULTS

The primary outcomes were the mean difference of glycated haemoglobin (%), fasting plasma glucose, and serum insulin as intervention effects. Secondary

outcomes included the measurement of cardiometabolic profile, antioxidative and oxidative stress status, nutritional status indices, and QoL. Tertiary outcomes involved the determination of safety and tolerability, and supplementation compliance.

CONCLUSION
The present clinical trial will reveal the effectiveness of multigrain supplementation among T2DM patients for the improvement of diabetes management.

Key Words: Beta-glucan; Clinical trial; Multigrain; Type 2 diabetes mellitus; Glycemic control

Core Tip: This is the first human clinical trial aimed to evaluate the effectiveness of multigrain supplementation among type 2 diabetes mellitus patients. The changes of glycemic control, cardiometabolic profile, oxidative stress status, nutritional status, and quality of life were measured. Our study also evaluated the safety, tolerability, and compliance of the supplementation.

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INTRODUCTION

Diabetes mellitus is one of the major global health problems and driving causes of morbidity and mortality around the world. Type 2 diabetes mellitus (T2DM) is a metabolic disease that causes sugar to build up in the bloodstream, characterized by insulin insensitivity as a result of insulin resistance in the muscle and adipose tissue, declining insulin production, and eventual pancreatic beta-cell failure[1]. When the beta-cells in the pancreas malfunction and/or insulin resistance develops in the liver, skeletal muscle, or adipose tissue, hyperglycemia arises, resulting in an excess level of glucose circulating in the blood[2]. T2DM has attained epidemic proportions worldwide with 415 million cases estimated globally in 2015, and the number is expected to increase dramatically in the next decades, reaching 642 million by 2040[3]. T2DM is the foremost common frame of diabetes mellitus, accounting for more than 90% of all cases of adult-onset diabetes mellitus in Malaysia[4]. According to the National Health and Morbidity Survey (2020)[5], one in every five adults in Malaysia has T2DM.

Uncontrolled diabetes mellitus may lead to microvascular complications (nephropathy, retinopathy, and neuropathy) and macrovascular complications, later leading to severe peripheral vascular disease, premature coronary artery disease, and increased risk of cerebrovascular diseases[6]. The main aim of diabetes management is targeted at reducing the acute and chronic diabetes complications, *via* the effective control of plasma glucose, blood pressure, lipid profile, and body weight concurrently[7]. The distinction between effective treatment and cure is obscured within the case of diabetes, but few individuals can reverse it through diet changes and be able to reach and maintain normal blood sugar levels without or with minimum medication. In particular, nutrition or dietary therapy is one of the trending complementary medicines, with the ultimate goal to control, prevent (occurrence), and reverse (by averting resulting complications after its onset) the disease[8].

Wholegrain is defined as consisting of the entire grain (bran, endosperm, and germ), and most fiber ingredient from the wholegrain is of insoluble origin, including the cellulose, hemicellulose, and lignin, with the exception of barley and oat (relevant sources of soluble fiber such as beta-glucan, pentoses, and arabinoxylan)[9]. Wholegrain is a good source of dietary fiber, resistant starch, antioxidants, and other important micronutrients, such as folic acid and other vitamins[10]. Fiber from the wholegrain has been shown to reduce the risk of T2DM by improving insulin sensitivity, lowering postprandial glucose response, and lowering inflammation[11]. In addition, laboratory and epidemiological investigations have reported that wholegrain, especially barley and oat, contain a high amount of beta-glucan, which has been proven to lower blood glucose levels, improve glucose tolerance, ameliorate hyperlipidemia, improve immunity, and decrease infections[12]. In parallel, the demand of the multigrain source in the commercial market is increasing tremendously due to an increased awareness of managing chronic diseases by ingesting health promoting functional foods[13]. Multigrain, a proper combination of few types of grains, could satisfy human body's need with essential nutritional benefits[14].

Several published clinical trials were looking into the effect of single grain supplementation on T2DM. Li *et al*[15] have conducted a clinical trial among overweight T2DM patients, and the results revealed that using oat as a therapeutic dietary regimen for 48 wk improved the body weight and glycemic control. The similar results have been inferred[16], where rice bran as a single treatment diet improved glycemic control and lipid profile in T2DM patients after 12 wk ingestion. In fact, multigrain consumption is more reflective towards human daily consumption. To date, study investigating the role of multigrain supplementation in T2DM patients is scarce.

Hence, the aim of this randomized clinical trial was to evaluate the effect of 12-wk of high beta-glucan multigrain supplementation on glycemic control in patients with T2DM. Secondary outcomes aim to evaluate the roles of the supplementation regimen for the amelioration of cardiometabolic health, antioxidative and oxidative stress, nutritional indices, and quality of life (QoL) among the T2DM patients. Tertiary outcomes involve the determination of safety and tolerability, and supplementation compliance.

MATERIALS AND METHODS

Study design and site

This was an open-label, randomized controlled trial, with an allocation ratio for the supplement (S) *vs* control (C) group at 1:1. All patients were registered T2DM patients. Study recruitment and enrollment began on October 14, 2020, and the completion date for enrollment was June 2021. The study site was the Day Care Clinic in Universiti Sains Malaysia Bertam Medical Center. The medical center serves as the referred medical facility in the northern region of Peninsular Malaysia.

Study population

The study population included 50 T2DM patients who were receiving standard diabetes care at Day Care Clinic. Patients aged at least 18 years of age, male or female, clinically diagnosed with T2DM for at least 6 mo duration without clinically manifest complications (retinopathy, nephropathy, neuropathy, vascular diseases, and food ulcer), and currently receiving pharmacological treatment with metformin or insulin, or a combination of metformin and glibenclamide were included in the trial (Table 1). Patients with gluten intolerance were excluded as the supplement contains gluten. Participants who have involved in another supplementary program were also excluded to avoid dilution effects.

Intervention groups

The study randomized all trial subjects into either group S or group C. Group S (n = 25) was supplemented with daily 60 g (2 sachets, 30 g each) of high beta-glucan (equivalent to 3.4 g) multigrain supplement for up to 12 wk. This multigrain supplement (Oat King®) was sponsored by TG Ocean Health Food Industries Sdn Bhd, Malaysia. It does not contain any food additives including food preservatives, coloring, flavoring, and sweetener. The main ingredients are oat, barley, brown rice, paddy, rice flour, corn flour, red kidney bean, black bean with kernel, and soybean (Table 2). Group S was required to consume the supplement two times per day (day and night). Patients were attending to the study site to receive and replenish the supplement at baseline, week 4, and week 8. All patients continued their standard medication as prescribed before the trial participation.

Group C (n = 25) continued the standard medication as prescribed prior to the trial. They were reminded not to alter their habitual dietary intake and physical activity level throughout the clinical trial period.

Study visits and measurements

Five categories of study visits have been adopted in this trial: Recruitment, screening, and inform consent form signing, randomization and blinding, enrolment visit, follow-up visits, and post week-12 visit. Figure 1 illustrates the trial flowchart.

Recruitment, screening and inform consent signing

All T2DM patients were invited face to face during their routine medical follow-up in the Day Care Clinic. Patient recruitment also occurred through electronic medical record review to identify potential participants. Patients were then invited for a screening session. The research team evaluated the eligibility criteria (both inclusion and exclusion criteria) and explained the research information in detail, followed by obtaining written inform consent. The research team did not coerce or unduly influence a patient to participate in the trial. Eligible patients underwent the randomization procedure.

Randomization and blinding

To generate a random allocation sequence, a computer-generated list of random numbers was used. Simple randomization at a 1:1 allocation ratio (1 group S: 1 group C) has been applied. The allocation sequence was concealed from the investigator enrolling and assessing participants on sequentially

Table 1 Inclusion and exclusion criteria for study population

Inclusion criteria	Exclusion criteria
Chronological age 18 years and above	Liver disease, kidney disease, or haematological disorders
T2DM ≥ 6 mo, stable regimen ≥ 6 mo without clinically manifest complications	Active gastric or duodenal ulcer
Male or female	Psychiatric disease or mental retardation
Pharmacological treatment with metformin or insulin, or a combination of metformin and glibenclamide	Cancer and other endocrine disorders
Free from antioxidant supplements	Alcohol or drug abuse
Free from anti-inflammatory supplements	Pregnancy or lactation
	Hormone replacement therapy
	Herbal remedies
	Gluten intolerance
	Currently under another supplementary program

T2DM: Type 2 diabetes mellitus.

Table 2 Active ingredients of Oat King®

Active ingredient	Scientific name	Percentage (%)
Oat	Avena sativa	11.80
Brown rice	Oryza sativa	9.55
Paddy	Oryza sativa	9.38
Rice	Oryza sativa	6.04
Corn	Zea mays	6.04
Red kidney bean	Phaseolus vulgaris	6.04
Black bean	Phaseolus vulgaris	6.04
Soy bean	Glycine max	5.17
Barley	Hordeum vulgare	4.98
Wheat	Triticum	4.98
Wheat germ	Triticum vulgare	4.98
Wheat bran	Triticum aestivum L	4.98
Coix seed	Coix-lacryma-jobi	4.22
Millet	Pennisetum glaucum	3.50
Red rice	Oryza longistaminata	2.46
Black rice	Zizania aqatica	2.46
Black sesame seed	Sesamum indicum	2.46
Navy bean	Phaseolus vulgaris	2.46
Mung bean	Vigna radiata	2.46

numbered, opaque, sealed, and stapled envelopes. To prevent subversion of the allocation sequence, the name and date of birth of the participant were written on the envelope. To randomize the participants, variables such as demographic data (age, gender, and ethnicity), clinical data (years of disease, glycemic status, and the presence of diabetic-related complications), physical activity, and medication (current prescribed medications) were taken into the consideration. To determine whether the patient would be randomized into the multigrain group S or C, randomization was made by reference to a statistical series based on the random sampling number drawn up by the statistician. The details of the series were unknown to any of the investigator or the coordinator. In order to implement blinding, participants

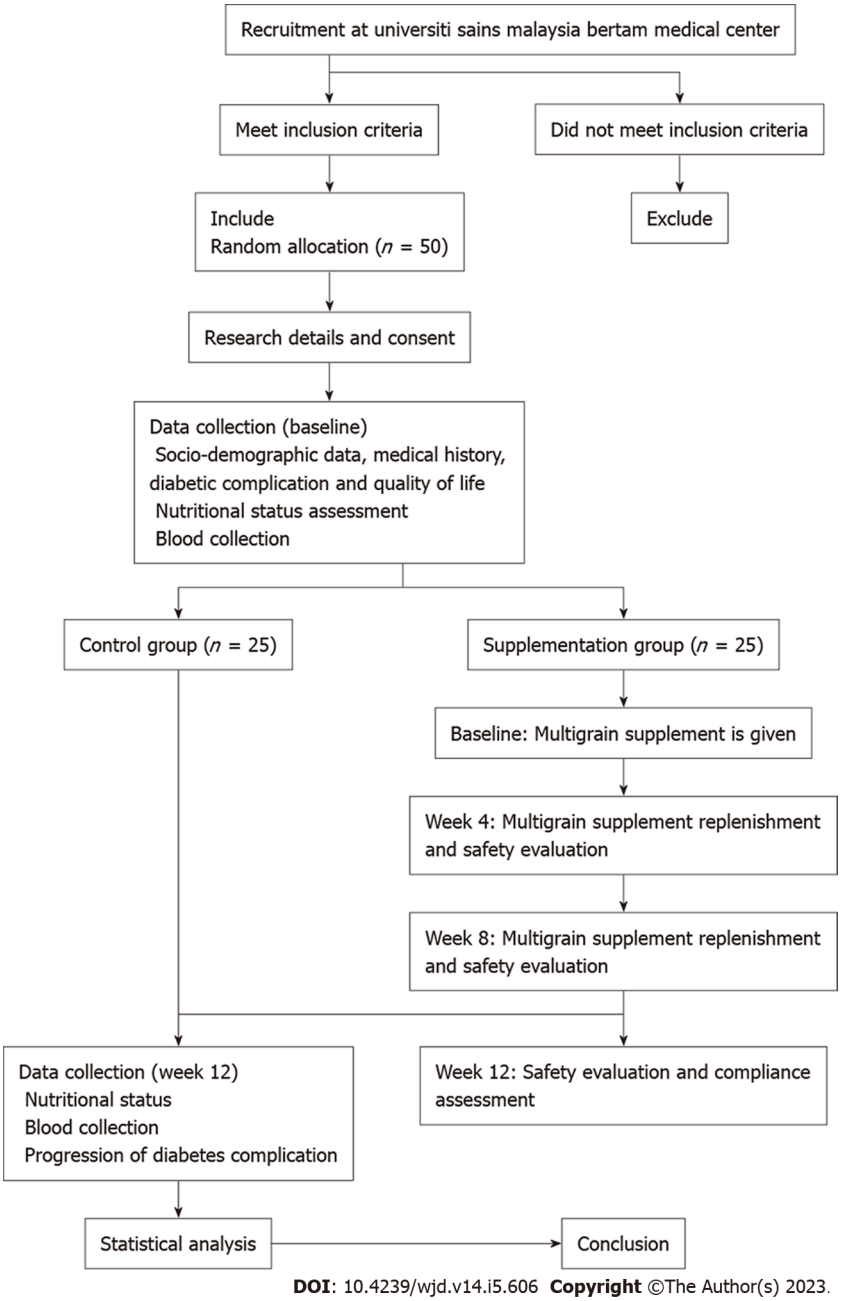

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Figure 1 Flow chart of the trial.

were notified individually of the assigned group S or C. However, only data collectors, the coordinator, and the medical officer in charge of the trial were aware of the allocated arm. Investigators, data analyst, and outcome adjudicator are kept blinded to the allocation.

Enrolment visit

The enrolment visit was consisted of a semi-quantitative questionnaire, physical examination, fasting blood sampling, and laboratory tests. The semi-quantitative questionnaire gathered information with regard to the socio-demographic background and medical history (including medical prescription). Lifestyle health behaviors included alcohol use, cigarettes smoking, and routine exercise practices. Physical examination involved the measurement of systolic and diastolic blood pressure, handgrip strength, and nutritional status assessments (anthropometry and body composition measurements).

A total of 20 mL of fasting venous blood was drawn from each participant for the subsequent clinical laboratory testing. Routine laboratory testing comprised of albumin, total protein and total bilirubin, urea, minerals, uric acid, and creatinine. Fasting plasma glucose, glycated haemoglobin, serum insulin, lipid profile, and liver and kidney function tests were performed. Upon centrifugation, serum and plasma samples were collected, and the antioxidative and oxidative stress statuses were assessed *via* the measurements of total antioxidant capacity, superoxide dismutase, glutathione, glutathione peroxidase

(GPx), malondialdehyde, protein carbonyl, and 8-deoxyguanosine concentrations.

Modified diabetes QoL-17 questionnaire[17] has been used to evaluate the changes of QoL, as assessed using 7 domains (physical functioning, role limitations due to physical health, role limitations due to emotional, energy fatigue, emotional well-being, social functioning, and general health).

The supplement group received the first month supply of multigrain supplement in the form of sachet. Detail use of the supplement was elaborated, and patients returned the used sachets packaging during the follow-up visits.

Follow-up visits

Patients were evaluated at 3 study visits (Figure 2) during the week-4, week-8, and post week-12 follow-ups at Day Care Clinic. At each follow-up, the evaluation of the safety, tolerability, and compliance to the multigrain supplementation was conducted. Adverse effects concomitant to the supplementation regimen, particularly the signs and symptoms of gastrointestinal discomforts, were recorded. Compliance to the supplementation was indicated as the recorded number of consumed sachets. Replenishment of multigrain supplement was implemented during week-4 and week-8 follow-ups, respectively. Disease progression in group C was evaluated following standard medication regimen. Both the supplement and control groups were reminded not to alter their routine dietary intake and physical activity level.

Post week-12 visit

After week 12, study questionnaire, physical examination, blood profile, and QoL assessments were performed in both the supplement and control groups. In-depth interviews have been conducted by the research team members among the patients in group S. The attitudes, positive and negative perceptions towards the supplementation, and perceived general health were interviewed. All study data was recorded into the case report form.

Power and sample size calculation

The results from a previous study[18] among T2DM patients were used to determine the trial sample size. The following formula is used to calculate the trial sample size:

$$n = \frac{2x\,[Z_{(1-\alpha/2)} + Z_{(1-\beta/2)}]^2}{\Delta^2}$$

Where n = sample size, Z = 0.8416 (for each arm, a setting of 80% power and 95%CI was used), Z = 1.96, and Δ^2 = mean difference or standard deviation. Thus, for this study, n = 18 subjects for each arm. With the consideration that the dropout rate was 20%, the needed sample size was 22 patients for each arm.

Statistical analysis

Data analysis in the form of intention to treat will be performed at the end of the study. All statistical analyses will be implemented using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, United States) software. The following statistical methods will be applied:

Assumptions will be checked for normality tests, and transformation will be applied as corrective procedures.

For descriptive statistics, categorical and continuous data, results will be presented as percentages, means with standard deviations, median and range.

For inferential tests, $P < 0.05$ will be used to indicate statistical significance (type I error) (two-tailed).

Analysis of the primary, secondary, and tertiary outcomes will be measured using Pearson's correlation, multivariate regression, repeated measures mixed models, logistic regression, and generalized linear models.

Ethics

The present study is conducted in accordance to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects have been approved by the Human Research Ethics Committee of Universiti Sains Malaysia (No: USM/JEPeM/20030183). Written consent is obtained from all patients, and the study has been registered in the clinical trial registry (ClinicalTrials.gov), with the registration ID: NCT04597229.

Study outcomes and measures

The patients' outcome measure has been assessed at two time points: Enrolment (baseline), and at the end of the treatment period (post week-12).

The primary outcomes were the changes in fasting plasma glucose, HbA1c, and serum insulin from enrolment to post week-12, and the differences in these changes between the two study arms.

Secondary outcomes include the measurement of lipid profile, liver function test and kidney function test comparing between the study groups. The change of nutritional status, antioxidative status, and oxidative stress biomarkers were assessed too.

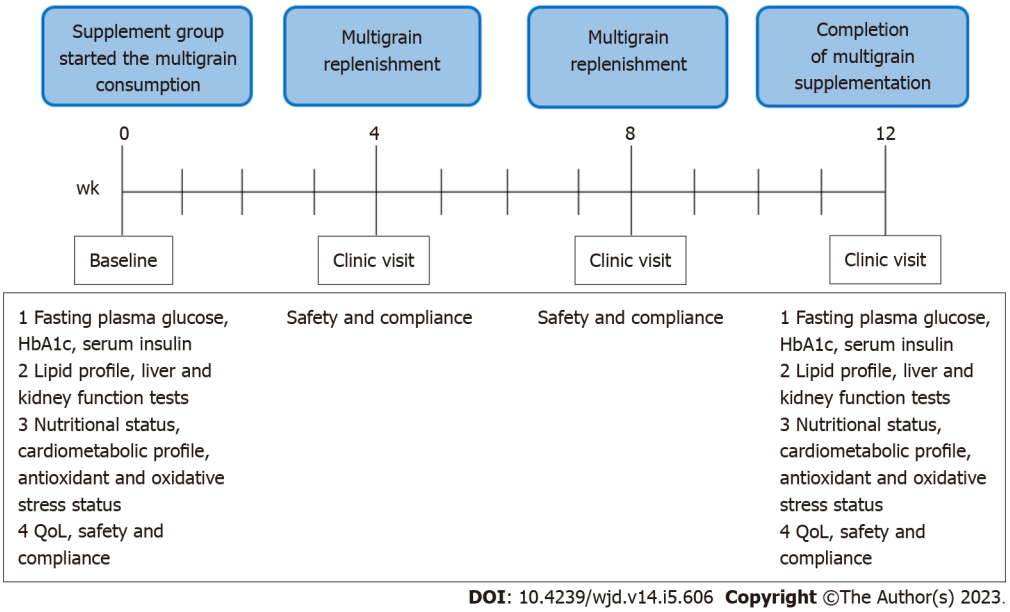

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Figure 2 Supplementation administration and follow-up assessment.

Tertiary outcomes were the change in QoL, and the difference in this change between the study groups. In term of safety evaluation, a list of gastrointestinal discomfort symptoms has been assessed among the participants in the supplementation group. The intensity of the gastrointestinal symptoms is defined as none, mild, moderate, severe, and very severe according to the symptoms (bloating, abdominal rumbling, flatulence, abdominal pain, nausea, vomiting, heart burn, loss of appetite, diarrhea, and constipation). Patients who showed symptoms have been referred to the physician in charge. Compliance to the supplementation regimen was assessed by counting the number of the consumed sachets during every follow-up visit (week-4, week-8, and week-12). Patients were asked to provide the reason for missed sachet consumption.

RESULTS

No result is provided as this is a pilot study protocol for a human clinical trial.

DISCUSSION

The current randomized control trial is aimed to evaluate the effects of multigrain supplementation as a complementary regimen *vs* a control (without supplementation) among patients with T2DM over a period of 12 wk. For the past decades, the underlying mechanisms for an association between grains and T2DM are not entirely clear, but grains may lower the risk of T2DM by improving insulin sensitivity[19]. Particularly, the potency of medium glycemic index multigrain flour to reduce glycemia in T2DM has been highlighted for the implementation of a better dietary plan for diabetes control[20]. Our study is designed to determine if multigrain supplementation, instead of single grain diet, is effective to ameliorate T2DM. Multigrain consumption is relatively a 'pure' dietary routine for human being.

Beta-glucan, pentose, and arabinoxylan are found in wholegrain fiber, especially in barley and oats, and other insoluble fibers, including cellulose, hemicellulose, and lignin[21]. These components play a vital role in a collective way, by improving the glycemic metabolism and reducing T2DM risk factor. Soluble fiber from oats and barley (with 3 g of beta-glucan intake per day) has been found to be effective in lowering total cholesterol and low-density lipoprotein (about 5% to 10% reduction, respectively)[15]. The latest finding also outlined the possible role of minimally processed whole grains over 2 wk in improving measures of glycemia in free-living adults with T2DM[22]. In addition, beta-glucan is evident to increase the intestinal viscosity, decrease the starch digestion, and reduce the food intake by increasing satiety, reducing hyperglycemia, lowering the lipid profile, and reducing weight[23].

Grains are generally high in magnesium. Magnesium is an essential co-factor for many enzymes, including the enzymes involved in glucose and insulin metabolism. Grain also contains a group of phenolic compounds, the avenanthramides. Avenanthramides are antioxidant and can enhance endothelial functions and anti-inflammatory properties[24]. Another potential antioxidant found in

grains is vitamin E. Vitamin E is an intracellular antioxidant, which prevents the oxidative damage of the polyunsaturated fatty acids in cell membranes. Vitamin E also facilitates to remain selenium in a reduced state[25]. Selenium plays an important role as a potent antioxidant. For example, GPx reacts with hydrogen peroxide to prevent harmful free radicals, DNA damage, and the formation of metabolic active carcinogens[26]. High selenium levels may help to reduce the formation of oxidized low-density lipoprotein (LDL) cholesterol and, as a result, reduce the risk of heart disease[27] and inflammation, strengthen the immune system in the body[28], and prevent the incidence of cancer[29]. Collectively, micronutrients in grains have their own beneficial roles to reduce the risk of T2DM complications.

In addition, bioactive compounds present in grains (such as phenolic compounds, phytosterols, betaine, and carotenoids) can help to improve insulin sensitivity and slow the progression of T2DM[30]. Bioactive compounds act by reducing the oxidative stress, inflammatory cytokine transcription, and subclinical inflammation[31] since increased oxidative stress seems to be a harmful component contributing to worsening insulin resistance and beta-cell dysfunction, which may lead to T2DM complications[32]. A previous study showed that a diet rich in polyphenols increased glucose tolerance and insulin sensitivity, and reduced the postprandial triglyceride response[33]. Moreover, phytosterols are known to be effective to reduce LDL cholesterol, as consumption of 2 g of plant sterol from wholegrain resulted in a 5.6% reduction in LDL cholesterol among T2DM patients after 4 wk ingestion [34]. Indirectly, this may reduce the risk of diabetes complications, particularly macrovascular complications.

Grain plays a significant role in reducing the energy intake. It has lower energy density, and the larger starch granules significantly contribute to a greater chewing rate, hence increasing satiation[10,35,36]. Fiber from the grain also increases gastric distension and delay the intestinal transit time, contributing to the stimulation of satiety signals[37] and increasing hormones levels involved in the energy homeostasis and plasma glucose control[38]. This process involves the stimulation of satiety signal in the brain, where body weight regulation hormones, ghrelin, peptide YY, cholecystokinin, gastric inhibitory polypeptide, and glucagon-like peptide 1 are regulated as part of the energy homeostasis and plasma glucose control[39]. This process might have a positive impact due to the change in gut microbiota profile[40,41] and cause a decrease in subclinical inflammation. Similarly, the slower process of carbohydrate digestion, as well as the glucose and free fatty acid absorption in the intestine[42], reduces insulin demand and stimulates fat oxidation, thus contributing to the reduction of fat storage[20]. Collectively, the synergistic mechanisms result in an increase in the hypothalamic satiety signal in the brain[20], which further leads to the body weight reduction and energy homeostasis, as well as glucose control[10,43-45].

Strengths of this study include a randomized controlled trial design, where the covariates could be equally distributed. The multigrain powder is formulated using commonly consumed grains, thus omitting the issues of food safety concern. Regular follow-up on a monthly basis allowed close monitoring of supplement adherence. The trial also included detail measurements of nutritional status, antioxidative status, oxidative stress biomarkers, and QoL, which allowed better result interpretation. These analyses will inform whether any potential effect extends to other metabolic or peripheral parameters. We acknowledge the small sample size of the study as the major limitation for this pilot clinical trial.

Important implications are expected from this research regardless of the findings. In a condition where beneficial effect is supported by evidence of a positive effect on long-term blood glucose levels, public health efforts should be undertaken to encourage the consumption of multigrain as functional foods. Contradictorily, if a beneficial effect is not supported, this could suggest that multigrain does not translate into strong long-term benefits for blood glucose control under daily conditions.

CONCLUSION

This is a pioneer, pilot clinical trial that aims to evaluate the efficacy of high beta-glucan multigrain supplementation among T2DM patients. Important trial outcomes, such as glycemic control, peripheral antioxidative capacity, cardiometabolic health, nutritional status, QoL, safety, and compliance have been studied extensively. The results of the trial are important to suggest a scientifically driven complementary dietary agent for better management of T2DM.

ARTICLE HIGHLIGHTS

Research background

Type II diabetes mellitus (T2DM) has emerged as a major public health challenge around the world. Diet is a major lifestyle factor that can greatly influence the incidence and progression of T2DM. The notion that foods not only provide basic nutrition but can also prevent diseases and ensure good health and longevity is now attaining greater prominence.

Research motivation

Typically, grains, with its rich non-starch polysaccharides content, are receiving concern among the scientific communities. Multigrain is rich with thiamine, riboflavin, pantothenic acid, iron, zinc, and copper, and it can be prepared using different preparation processes, which usually comprises a high amount of dietary fiber content. Multigrain consumption is indeed a more representative dietary intervention as compared to single grain intake. There is a need to examine whether supplementation with multigrain, a more representative dietary regimen to human routine consumption pattern, would yield better outcomes among T2DM patients.

Research objectives

The objectives of the present study were to evaluate the effects of multigrain supplementation on glycemic control, cardiometabolic profile, oxidative stress, nutritional status, and quality of life (QoL) among T2DM patients. The safety, tolerability, and adherence of the supplementation were evaluated.

Research methods

Fifty T2DM patients have been randomly assigned to receive either 60 g multigrain supplementation (containing 3.4 g beta-glucan) coupled with prescribed standard medication regimen (n = 25), or standard medication regimen alone (n = 25) for 12 wk. Study outcomes involved the changes of glycemic control, cardiometabolic profile, oxidative stress, nutritional status, and QoL.

Research results

No result is provided as this is a pilot study protocol for a human clinical trial.

Research conclusions

This is a pioneer, pilot clinical trial that aims to evaluate the efficacy of high beta-glucan multigrain supplementation among T2DM patients. Important trial outcomes, such as glycemic control, peripheral antioxidative capacity, cardiometabolic health, nutritional status, QoL, safety, and compliance, have been studied extensively. The results of the trial are important to suggest a scientifically driven complementary dietary agent for better management of T2DM.

Research perspectives

The findings are expected to contribute and expand the fundamental mechanism of the role of multigrain as a complementary management agent in diabetic physiology.

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FOOTNOTES

Author contributions: Mohd Ariffin NA, Mohd Sopian M, and Lee LK were responsible for the study conception and design, data acquisition, analysis, and interpretation, and manuscript drafting; Mohd Ariffin NA and Lee LK critically reviewed and revised the article for important intellectual content of the manuscript; all authors reviewed and approved the final version of the manuscript to be published.

Institutional review board statement: The study was reviewed and approved by the Human Research Ethics Committee of Universiti Sains Malaysia (No: USM/JEPeM/20030183).

Clinical trial registration statement: This study is registered in the clinical trial registry (ClinicalTrials.gov), with the registration ID: NCT04597229.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrolment.

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SYSTEMATIC REVIEWS

Cardiometabolic effects of breastfeeding on infants of diabetic mothers

Reem Elbeltagi, Mohammed Al-Beltagi, Nermin Kamal Saeed, Adel Salah Bediwy

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Abstract

BACKGROUND

Breast milk is the best and principal nutritional source for neonates and infants. It may protect infants against many metabolic diseases, predominantly obesity and type 2 diabetes. Diabetes mellitus (DM) is a chronic metabolic and microvascular disease that affects all the body systems and all ages from intrauterine life to late adulthood. Breastfeeding protects against infant mortality and diseases, such as necrotizing enterocolitis, diarrhoea, respiratory infections, viral and bacterial infection, eczema, allergic rhinitis, asthma, food allergies, malocclusion, dental caries, Crohn's disease, and ulcerative colitis. It also protects against obesity and insulin resistance and increases intelligence and mental development. Gestational diabetes has short and long-term impacts on infants of diabetic mothers (IDM). Breast milk composition changes in mothers with gestational diabetes.

AIM
To investigate the beneficial or detrimental effects of breastfeeding on the cardiometabolic health of IDM and their mothers.

METHODS
We performed a database search on different engines and a thorough literature review and included 121 research published in English between January 2000 and December 15, 2022, in this review.

RESULTS
Most of the literature agreed on the beneficial effects of breast milk for both the mother and the infant in the short and long terms. Breastfeeding protects mothers with gestational diabetes against obesity and type 2 DM. Despite some evidence of the protective effects of breastfeeding on IDM in the short and long term, the evidence is not strong enough due to the presence of many confounding factors and a lack of sufficient studies.

CONCLUSION
We need more comprehensive research to prove these effects. Despite many obstacles that may enface mothers with gestational diabetes to start and maintain breastfeeding, every effort should be made to encourage them to breastfeed.

Key Words: Breast milk; Breastfeeding; Gestational diabetes mellitus; Cardiometabolic effects; Infants of diabetic mothers; Obesity

Core Tip: Breast milk is the ideal nutritional source for all neonates. It protects against many cardiometabolic disorders for babies and their mothers in the presence or absence of gestational diabetes. It protects against overweight, obesity, insulin resistance, prediabetes, diabetes, and metabolic syndrome in offspring regardless of gestational diabetes status. Therefore, it prevents significant risk factors predisposing to cardiovascular diseases during childhood and adulthood. Every effort should be made to encourage breastfeeding.

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INTRODUCTION

Breast milk is the best and principal nutritional source for neonates, providing them with the needed protein, fat, carbohydrate, vitamins, and minerals requirements. In addition, it provides them with different substances and bioactive agents that help protect them against infections and inflammation by contributing to a healthy microbiome, organ development, and an efficient immune system[1]. Breast milk is rich in growth factors that support the development and growth of the newborn's brain, gut, endocrine, and vascular systems[2]. Many studies suggested that breast milk protects infants against many metabolic diseases, predominantly obesity and type 2 diabetes[3]. Breast milk is continuously changing with dynamic and bioactive composition modification from colostrum to late stages of lactation. It often varies diurnally, within feeds, between different populations, and even between mothers from the same population to meet the metabolic needs of their babies[4]. The amount of breastmilk needed at one month of age is about 650 mL/d, increased to 770 mL/d at three months and 800 mL/d at six months, then dropped to 520 mL/d by one year of age. In addition, the duration and frequency of breastfeeding also change with infant development and maturation, starting with 20 to 40 min, up to six times/d, which is reduced to 10-20 min when the infant reaches three months of age. The frequency of breastfeeding decreases as the weaning starts[5].

Diabetes mellitus (DM) is a chronic metabolic and microvascular disease that affects all the body systems and all ages from intrauterine life to late adulthood. DM that occurs during pregnancy could have its onset before or arise as de novo for the first-time during pregnancy (gestational DM), which could disappear or persist after delivery[6]. Impaired glucose tolerance occurs in 3%-10% of pregnancies and correlates positively with the average diabetes incidence in the general population. The risk of

gestational diabetes increases with advanced maternal age, obesity, non-white ancestry, and physical inactivity[7]. Gestational diabetes has short and long-term effects on infants of diabetic mothers (IDM). Neonates have a higher risk of post-natal hypoglycemia, macrosomia, respiratory problems, hypertrophic cardiomyopathy, congenital disabilities, and various metabolic and hematologic disorders. At the same time, there is an increased risk of obesity during childhood and type 2 DM in adulthood[8]. Breastfeeding is well known to have many beneficial effects on both mothers and infants. However, the breast milk of mothers with diabetes has altered composition. Therefore, it is expected to have different effects than those from non-diabetic mothers[9]. This review investigates the beneficial or detrimental effects of breastfeeding on the cardiometabolic health of IDM and their mothers.

MATERIALS AND METHODS

Literature search

To establish an evidence-based vision of this aim, we performed a thorough literature review by searching the available electronic databases, including Cochrane Library, PubMed, PubMed Central, Cumulative Index to Nursing and Allied Health Literature, Embase, Web of Science, Library and Information Science Abstracts, Scopus, and the National Library of Medicine catalog up until December 15, 2022, using the keywords: Diabetes Mellitus, Gestational Diabetes, Cardiometabolic, Breastfeeding, Breast milk. We identified 1363 articles, 98 of which were removed due to duplication. After the screening of the titles and abstract, we excluded 1016 articles. From the remaining 249 full-text articles, only 121 articles fulfilled the eligibility criteria.

We included full-text research articles (72 articles), metanalysis (13 articles), systematic reviews (5 articles), reviews (29 articles), and Case reports (2 articles). We included articles that were written in English and concerned with the effects of breastfeeding on the cardiometabolic effects in IDM. Figure 1 shows the study flow chart. Reference lists were checked, and citation searches were performed on the included studies. We also reviewed the articles that are available as abstracts only. We excluded articles with a commercial background.

RESULTS

Most of the literature agreed on the beneficial effects of breast milk for both the mother and the infant in the short and long terms. Breastfeeding protects mothers with gestational diabetes against obesity and type 2 DM. Despite some evidence of the protective effects of breastfeeding on IDM in the short and long term, the evidence is not strong enough due to the presence of many confounding factors and a lack of sufficient studies.

DISCUSSION

Beneficial effects of breastfeeding

Breast milk is the ideal nutrition source for the infant, especially in the first six months, as it provides the baby with everything they need in the proper proportions for the first six months of life. Its composition modifies according to the infants' changing requirements, particularly in the first few weeks of life. Colostrum is the wonder of breastfeeding in the first post-natal days, with thick yellowish color, high protein, low sugar, and many beneficial compounds. It helps develop the baby's immature gut to be ready to receive the increasing amount of breastfeeding in the following days[1]. In addition, early breastfeeding in the delivery room may prevent the development of post-natal hypoglycemia in IDMs [10]. However, breastfeeding has low vitamin D. Breastfed babies should be supplemented with vitamin D[11]. The low iron profile in breast milk could be beneficial in decreasing the risk of bacterial growth. Iron supplementation in breastfed babies should be considered to improve brain and cognitive development, especially those born prematurely or at low birth weight [12].

Breastfeeding performs crucial effects on the programming activity during early life. Many recent meta-analyses of several studies provide strong evidence that breastfeeding benefits neonates, infants, children, and lactating mothers considerably. The degrees of these beneficial effects vary according to different settings' background environmental and hygienic conditions[13]. According to 28 metanalyses, breastfeeding protects against infant mortality, especially in low-income settings, by 4-10 times and by 36% in high-income settings[14]. Breastfeeding also protects against many diseases, such as diarrhea by 75% and respiratory infections by 57%, particularly in young children[15]. Breast milk has plenty of antibodies that protect the baby against many viruses and bacteria, which is particularly important during the early critical months of life. Colostrum provides the baby with many different antibodies, especially immunoglobulin A, a crucial element of the baby portal immunity, protecting the nose,

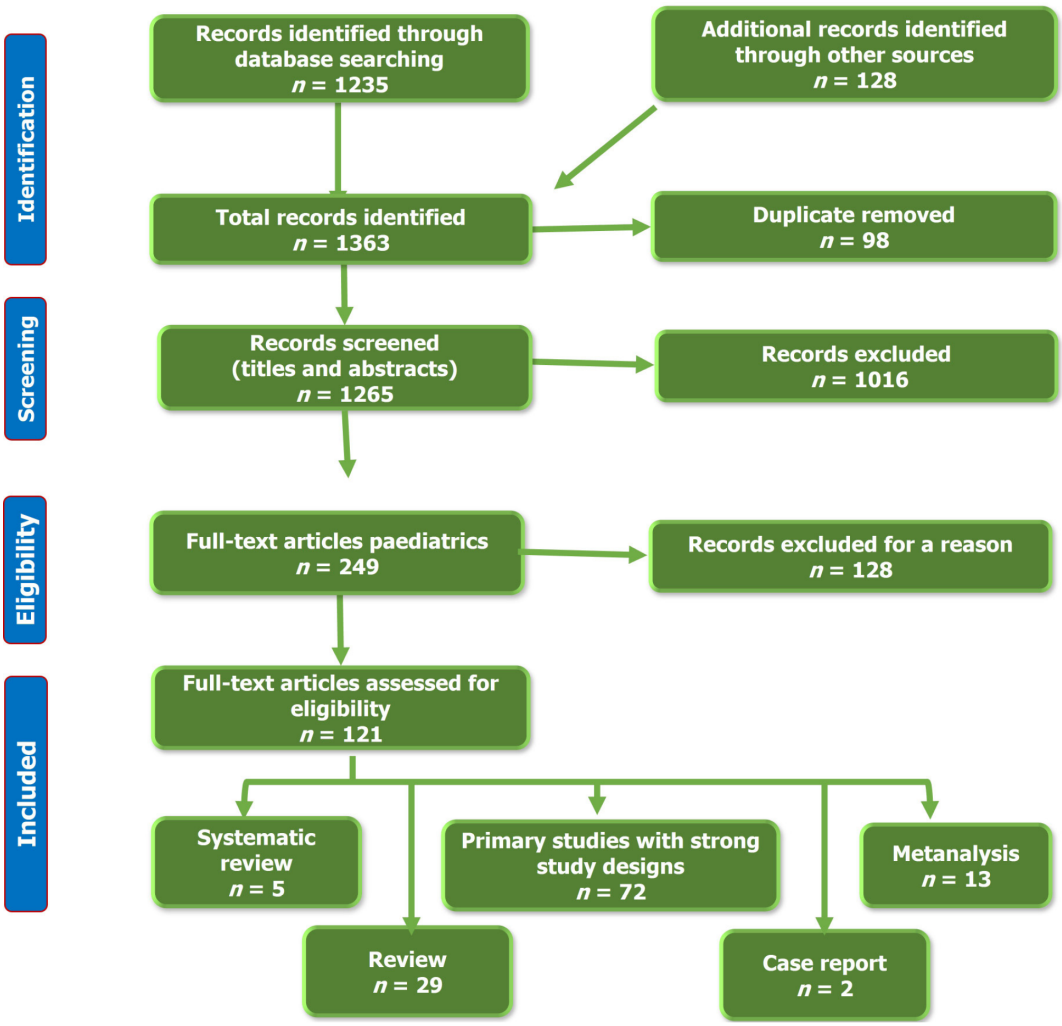

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Figure 1 The flow chart of the included studies.

throat, and gastrointestinal tract[16].

Breastmilk may also give some protection against eczema, allergic rhinitis, asthma, and food allergies, but with weak evidence[17]. Even though breastfeeding protects against malocclusion and dental caries, more prolonged breastfeeding (beyond one year of age) and nocturnal breastfeeding increase dental caries by two to three folds[18]. It can decrease the incidence of necrotizing enterocolitis in preterm babies. A meta-analysis by Altobelli *et al*[19] showed that premature infants who received both their own and donated breastmilk had a statistically significant reduced risk of necrotizing enterocolitis, possibly due to the reduced microbial contamination, its pre, and probiotic effects, and its unique immunological components. Breastfed infants are less liable to develop Crohn's disease and ulcerative colitis. A meta-analysis by Xu *et al*[20] showed that breastfeeding is associated with a reduced risk of Crohn's disease and ulcerative colitis in all ethnicities, particularly among Asians. Breastfeeding has dose-dependent protection against Crohn's disease and ulcerative colitis, with the most potent effect when breastfeeding continues for at least one year.

Breastfeeding causes a mild reduction of body mass index (BMI) without significant differences in growth outcome. However, a meta-analysis by Giugliani *et al*[21] showed a 13% reduction in the risk of later obesity. Grube *et al*[22] showed that breastfeeding for longer than four months significantly reduces the risk of developing overweight and obesity than in non-breastfed babies or those with a shorter breastfeeding period. The weight-reducing effects of breastfeeding could be related to the development of specific strains of gut microbiota that could impact fat storage[23]. At the same time, breast milk contains more leptin hormone than formula milk, if present. Leptin is a vital hormone that regulates the baby's appetite and controls fat storage[24]. Meanwhile, breastfed infants have more self-regulation of their feeding habits, especially those in on-demand feeding, which supports them in developing healthy feeding patterns[25].

Therefore, the risk of type 2 DM can be reduced by 24% to 32% and to a lesser degree with type 1 DM [26]. Meanwhile, six months or longer of breastfeeding decreases the risk of childhood leukemia by 14%-20%[27]. Breastfeeding also helps to alleviate the clinical course and the severity of urolithiasis

identified during infancy. Infants who had prolonged breastfeeding are more liable for reduced size and/or the number of urinary stones. Infants receiving breastfeeding for the first six months need less treatment and have less growth impairment[28].

There is also an association between breastfeeding and increased intelligence by at least 2-3 points after adjusting for the home environment and average parental intelligence quotient (IQ). This increase may be related to the nutritional components, non-nutritional bioactive factors, maternal-infant bonding with physical intimacy, interactions, touch, and eye-to-eye contact. Infants with breastfeeding are less liable to have behavioral problems or learning difficulties than bottle-feeding infants. This breastfeeding-promoting effect on the baby's optimal brain development during early life can have long-lasting impacts on infant neurodevelopmental function[29,30]. This effect is more pronounced in preterm babies than in term infants. Belfort *et al*[31] showed that predominant breastfeeding in the first four weeks of life is related to a larger volume of deep nuclear gray matter volume at term equivalent age and improved IQ, working memory, academic achievement, and motor function at the age of seven in the very preterm infants. Increasing breastfeeding duration is positively correlated with enhancing cognitive development. Ribas-Fitó *et al*[32] observed a linear dose-response relationship between breastfeeding and cognition at the age of four in children with a history of antenatal exposure to dichlorodiphenyltrichloroethane (DDT) despite the risk of breastfeeding pollution with DDT. Breastfeeding protects against indoor and outdoor air pollution exposure and adverse outcomes due to the effects of long-chain polyunsaturated fatty acids (LC-PUFA), carotenoids, antioxidant vitamins, flavonoids, cytokines, and immunoglobins. Though breastfeeding may be polluted with many pollutants, its protective effects outweigh its potential health hazards to the infant[33] (Table 1).

Beneficial effects on lactating mothers

Breastfeeding has significant impacts on lactating mothers. It may help overweight mothers to lose weight. Numerous studies described a positive correlation between postpartum weight loss and breastfeeding, while others studied did not find a significant association. Several possible mechanisms, determinants, and metabolic pathways may play a role in this weight reduction[34]. Lactating mother burns about 20 calories/ounce of breastmilk she produces. Therefore, one day of breastfeeding may help burn up to 900 calories and more fat. Jarlenski *et al*[35] showed that exclusive breastfeeding for at least three months or more has a minimal but considerable effect on postpartum weight reduction among American women. Schalla *et al*[36] showed that returning to a pre-pregnancy body shape is an important feature that encourages mothers to continue breastfeeding. One of the other immediate benefits that lactating mothers have with breastfeeding is the rapid involution of the gravid uterus to return to its pre-gravid size due to oxytocin release in response to the sucking of the breastfed baby, which boosts uterine contractions and lessens bleeding. In addition, oxytocin helps to increase maternal-infant bonding[37].

Breastfeeding is correlated with a significantly reduced risk of ovarian cancer in general and, in particular, for the most lethal high-grade serous subtype of ovarian cancer. This finding suggests that breastfeeding is a possibly modifiable factor that may decrease the risk of ovarian cancer regardless of the effect of pregnancy. The longer the breastfeeding duration, the more the risk is reduced[38]. A meta-analysis by Unar-Munguía *et al*[39] showed that exclusive breastfeeding significantly reduces breast cancer risk compared to non-breastfeeding parous women. Breastfeeding mothers are less likely to suffer postpartum depression than mothers who do not breastfeed or discontinue it early. These effects are maintained for the first four postpartum months. Conversely, postpartum depression reduces the breastfeeding rate in a reciprocal mechanism[40].

The longer the duration of breastfeeding, the less the risk of developing type 2 diabetes in lactating women. Schwarz *et al*[41] showed that breastfeeding is associated with improved maternal glucose metabolism. They also showed an increased risk of developing type 2 diabetes in later life when the parous women lactate for less than a month after term pregnancy, regardless of the women's BMI or physical activity. Breastfeeding also decreases the risk of hypertension, hypercholesteremia, and arthritis. In addition, breastfeeding protects mothers who breastfeed their children for five months or more in at least one pregnancy against coronary artery disease (CAD), with a 30% risk reduction later in life. Conversely, parous women who never breastfed or stopped breastfeeding early have a two-fold increased risk of CAD[42].

Mechanism of protective effects of breastfeeding

Many possible mechanisms are proposed to explain the protective effects of breastfeeding. These mechanisms include the beneficial effects of breastfeeding on the respiratory, nervous, and immune systems, which are related to breast milk's anti-inflammatory, antioxidant, neuroprotective, and immunomodulatory features[33]. The high cholesterol content of breast milk during infancy inversely suppresses endogenous cholesterol synthesis in adulthood by suppressing the regulation of hydroxymethyl-glutaril liver coenzyme A[43,44]. Therefore, breast milk protects against the development of hypercholesteremia, especially low-density lipoprotein cholesterol which is a significant risk factor for coronary heart diseases[45]. This cholesterol-regulating effect of breast milk can explain its protective effects against atherosclerosis, hypertension, and coronary heart diseases. The low sodium and the high LC-PUFA contents of breast milk compared to formula milk might give more protection against the

Table 1 Beneficial effects of breastfeeding

Beneficial effects of breastfeeding
Neonates
It helps the development of the immature gut
It bears immunological, nutritional, and neurodevelopmental benefits for preterm neonates
It decreases the incidence of necrotizing enterocolitis in preterm babies
It prevents the development of post-natal hypoglycemia in IDMs
It improves neonatal portal immunity, *e.g.*, nose, throat, and gastrointestinal tract
Infants and children
It is the ideal exclusive food for the first six months and the main food till 12 mo of age
It decreases infant mortality and protects against sudden infant death syndrome
It is crucial for the infant's eyesight, speech, jaw, and mouth development
It increases intelligence by at least 2-3 points
It promotes healthy weight gain in infants
It reduces diarrhea and respiratory infections
It protects against common childhood allergic diseases; *e.g.*, eczema, allergic rhinitis, asthma, and food allergies
It protects against Celiac disease
It protects against malocclusion and dental caries
It decreases the incidence of inflammatory bowel diseases, *e.g.*, Crohn's disease and ulcerative colitis
It reduces body mass index and the risk of later obesity
It reduces the risk of type II DM by 24% to 32% and, to a lesser degree, type I DM
It decreases the risk of childhood leukemia by 14%-20%
It decreases the severity of urolithiasis during infancy
Lactating mothers
It helps rapid involution of the gravid uterus to return to its pre-gravid size
It decreases the risk of postpartum bleeding
It increases maternal-infant bonding
It significantly reduces postpartum depression in the first four postpartum months
It helps overweight mothers to lose weight
It significantly reduces the risk of ovarian cancer, especially the most lethal high-grade serous subtype of ovarian cancer
It significantly reduces breast, endometrial, and thyroid cancer risk
It reduces the risk of developing type 2 diabetes
It decreases the risk of hypertension, hypercholesteremia, and coronary artery disease
It decreases the risk of osteoporosis and arthritis

IDM: Infants of diabetic mothers

future development of hypertension during childhood and adulthood[46,47]. LC-PUFA is a crucial element of the tissue membrane system, such as the coronary endothelial system, therefore reducing the risk of coronary heart disease and stroke during adulthood[48]. Breastfeeding can also reduce fasting insulin and insulin resistance in infancy, childhood, and adulthood[49,50].

Breastfeeding has a behavioral modifying effect on the infant's appetite, satiety, and feeding pattern due to its unique micro- and macro-nutrients and hormonal contents. These unique features of breastmilk explain its protective role against obesity[51,52]. Breastmilk contains leptin, which is not present in formula milk, and less protein and fat than formula milk, so breastfeeding is likely to adequately stimulate the secretion of insulin growth factor-type 1. Subsequently, it can induce adequate insulin secretion, fewer adipocytes stimulation and size, and balancing fat reserve, which eventually

results in adequate weight gain and is less likely to cause overweight and obesity[51,53]. Breastmilk can also modulate the expression of obesity-predisposing genes, preventing the development of obesity and other non-communicable diseases[54]. Breastmilk has a significant regulating effect on the blood glucose level due to its high content of LC-PUFA. The LC-PUFA amount in the skeletal muscle membranes is inversely proportional to the fasting blood glucose level, insulin resistance, subsequent hyperinsulinemia, and type 2 diabetes[55,56]. The low protein content, the lower volume of breastmilk consumed by the infant, and the differences in the levels of hormones of insulin, neurotensin, intro-glucagon, motilin, and pancreatic polypeptide, and lower subcutaneous fat deposition are additive protective factors against developing type 2 diabetes[57].

Breastfeeding has well-known immune-modulating and protective effects against many immune and allergic disorders, especially in low-income countries[58]. Breastfeeding supports passive and active immunity in infants and young children[59]. Breastfeeding also protects against many infectious diseases in infancy and early childhood. These protective effects are dose-dependent, increasing with exclusive breastfeeding and more prolonged duration[60]. These protective and immune-enhancing effects of breastfeeding are due to its richness of many compounds that enhance both innate (such as various cellular components, lysozyme, oligosaccharides, lactoferrin, the cluster of differentiation 14, and probiotics components)[61-63] and active (such as immunoglobins A, M, and G) immunity[59]. In addition, breastmilk contains many immune-modulating ingredients, such as cytokines or nutritional components, such as LC-PUFA; vitamins A, B12, and D, and zinc[58]. Omega-3 LC-PUFA, abundant in breast milk, helps T-cell membrane stabilization, T-cell signaling, improvement, and reduction of many pro-inflammatory substances production. On the contrary, omega-6 LC-PUFAs stimulate their production[64]. Exclusive breastfeeding modulates the inflammatory status by promoting an anti-inflammatory cytokine milieu and decreases gut inflammation that persists throughout infancy, adolescence, and adulthood[65-67]. The anti-inflammatory effect of breastfeeding is due to the presence of various immunoreactive and immunomodulator factors such as lactoperoxidase, lactoferrin, immunoglobins, osteopontin, superoxide dismutase, platelet-activating factor acetylhydrolase, alkaline phosphatase, antioxidant compounds, bioactive factors, and many growth factors that have anti-inflammatory effects[62].

The anti-inflammatory and immune-modulating effects of breastmilk boost lung development and function. In addition, the breastmilk cytokines, growth factors, and maternal immunoglobins may stimulate lung growth and development, inhibit airway inflammation, and decrease the risk of developing asthma. Breastfeeding is also associated with a reduced risk of being overweight and obese and, consequently, better lung function[68-70]. Breastmilk nutrients such as β-carotene, lutein/zeaxanthin, polyphenol, and anthocyanin also affect lung efficiency[71]. Breastfeeding effects on DNA methylation provide an additional protective mechanism for the respiratory tract and improve lung development and maturation[72]. Moreover, sucking during breastfeeding stimulates the development of the diaphragm and the respiratory muscles, enhances the coordination between swallowing and respiration, and, thus, improves lung capacity[73].

The better structural and physiological neurodevelopment and cognitive and psychomotor performance associated with breastfeeding are related to many factors. Breast milk is rich in LC PUFAs, antioxidants (such as carotenoids and flavonoids), and other nutrients and bioactive factors that can induce immunomodulation and reduce oxidative stress and neuroinflammation[29,71,74]. Breast milk also contains many compounds essential for proper brain development, neurotransmitters synthesis, synaptogenesis, and intracellular communication. Breast milk is rich in LC PUFAs, glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor (BDNF), gangliosides, sialic acid, lutein, choline, zeaxanthin, and flavonoids. These nutrients are essential in the human brain's gross and functional development[75-77]. Other social and environmental factors associated with breastfeeding, such as mother-infant bonding and educational and socioeconomic levels, may also play a role in better neurodevelopment[78].

Metabolic effects of breastfeeding

Effects on the mothers: Breastfeeding induces more favorable metabolic parameters in lactating women. It initiates a metabolic shift from pregnancy to postpartum with the alteration of resource allocation from the caloric storage stage to the milk production phase with lipid transport facilitation to the mammary gland to help in milk synthesis[79]. Stuebe[80] showed that early, high-intensity breastfeeding might help to reset the endocrine balance to shift from the insulin-resistant state in pregnancy to insulin sensitive state; thus, lactation may protect against long-term cardiometabolic health consequences. Breastfeeding induces improved glucose utilization through reduced insulin production, enhanced insulin sensitivity, and decreased β-cell proliferation[81]. Therefore, lactating women are less liable to have atherogenic blood lipids and have better glucose and lipid metabolism, lower fasting and postprandial blood glucose, low insulin levels, and more insulin sensitivity than non-lactating women, especially in the first four postpartum months[82]. In addition, lactation reduces the risk of obesity, metabolic syndrome, cardiovascular diseases, and type 2 diabetes during mid to late life[83,84]. The liver, white adipose tissues and skeletal muscles are responsible for about 50% of the mammals' metabolic rate. Breastfeeding increases hepatic mitochondrial respiration, therefore increasing the metabolic rate. In a study of rats, Hyatt *et al*[81] showed that lactation induces PPARδ protein level

changes in the liver, white adipose tissue, and skeletal muscle, which may partially clarify the observed lower blood glucose levels. A large study from Japan showed that the longer the duration of breastfeeding, the less risk for developing metabolic syndrome in women under 55 years of age[85]. The long-term protective effects of breastfeeding were also confirmed by Wiklund *et al*[86], who showed that breastfeeding longer than six months gives protection against obesity, impaired glucose tolerance, insulin resistance, hypercholesteremia, and hypertension that could persist for 16 to 20 years later.

Effects on the infants: Breastfeeding has significant effects on infant metabolism through different mechanisms. Breast milk has numerous beneficial compounds that can cause epigenetic changes in genes that control metabolism or predispose to insulin resistance, diabetes, or obesity. For example, breastmilk downregulates phosphatase and tensin homolog and acetyl-CoA carboxylase beta genes, protecting against developing insulin resistance and DM[87-89]. Meanwhile, breastfeeding appears to counter the deleterious effect of the peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism on anthropometrical parameters in adolescents[90]. The liver X receptors gene expression is also modulated by breastfeeding. Activating these receptors stimulates a set of target genes needed for the de novo synthesis of triglycerides and cholesterol transport in many tissues[91]. In addition, infant serum lysophosphatidylcholine, which is positively associated with obesity risk, is affected by breast milk fatty acids composition and, interestingly, milk protein content and composition in early but not late lactation[92]. Therefore, breastfed infants are metabolically different from the infant formula regarding the lipid and energy metabolism levels (ketone bodies, carnitines, and Krebs cycle)[93]. Breastfeeding positively affects metabolic variables, anthropometric indices, and diabetes-prompting genes compared to bottle feeding[87]. Breastfeeding also increases BDNF, which enhances synaptogenesis and neuronal development in infants between 4-6 mo of age[94]. This neurotrophic factor impacts numerous metabolic pathways by modifying the hypothalamus or specific neurotransmitters that facilitate food intake[95]. The effects of breastfeeding on the infant metabolism are dose-dependent. Corona *et al*[96] showed that the duration of breastfeeding is inversely related to the Z-score of triceps skinfold-for-age till the age of three years. On the other hand, Martin *et al*[97] showed that even with a long duration, exclusive breastfeeding failed to reduce insulin resistance or cardiometabolic risk parameters at 11.5 years. Therefore, the breastfeeding effect on the body's metabolism still demands additional analysis and research. We need to study why there are differences in the results of these studies and confounding factors that may impact their results.

Changes in breast milk composition with DM

Breast milk is a biologically-active, continuously dynamic fluid that significantly differs from woman to woman and from one stage to another. It is affected by various maternal factors such as term-preterm labor, maternal diet, metabolic disorders, and diseases[2,98]. DM is a chronic systemic metabolic disorder that could affect pregnant ladies with pregestational or gestational (a de novo) onset[99]. Mother with gestational DM has a 15 to 24 h delay in lactogenesis II (initiation of lactation) markers such as citrate, lactose, and total nitrogen to reach levels similar to healthy women[100]. This delay in breast milk initiation in women with gestational diabetes could be related to low levels of circulating human placental lactogen in the latter stages of pregnancy, which is positively correlated with mammary gland growth during pregnancy[101].

In addition, Arthur *et al*[102] and Azulay Chertok *et al*[103] found a significant delay in the timing of the lactose increase in the colostrum in lactating women with type 1 or gestational DM, accompanied by a reduced milk volume in the first three postpartum days, as lactose is the main osmotic ingredient in the human milk. The observed delay in citrate concentration rise in colostrum may cause a delay in the de novo medium-chain fatty acids synthesis, as citrate is essential for acetyl CoA generation from glucose[104]. Avellar *et al*[105] showed that women with gestational DM had higher colostrum contents of cytokines and chemokines, with increased levels of interleukin 6 (IL-6), IL-15, interferon-γ, reduced IL-1ra levels, and a decreased granulocyte-macrophage colony-stimulating factor (GM-CSF), causing altered immune composition of the colostrum. Bitman *et al*[106] found that women with type 1 DM who started to pump milk at 72 h postpartum firstly gave breast milk with reduced total fat, medium-chain fatty acids, and total cholesterol but increased linoleic, oleic, and polyunsaturated long-chain fatty acid content than healthy women. These fatty acid profile changes are related to changes in specific endogenous metabolic pathways[107]. Women with type 1 DM also have impaired mammary gland lipid metabolism and high glucose and sodium contents in mature milk. However, no significant differences exist in the free amino acid profile in women with and without gestational DM[108]. The high amino acid levels in the colostrum and high levels of saturated and non-saturated fatty acid levels in mature milk in lactating women with and without gestational DM are crucial for neonatal development in the early period of life[109]. In addition, Suwaydi *et al*[110] showed that gestational DM has significant relationships with metabolic hormone concentrations, including ghrelin, insulin, and adiponectin. However, these relationships might be restricted to the early lactation stage.

Cardiometabolic protective effects of breastfeeding on diabetic mothers and their offspring

Breastfeeding is associated with reduced risk of type 2 DM in women with gestational DM for up to two postpartum years. In addition, breastfeeding may have long-lasting protective effects beyond two years

after delivery, especially with greater lactation intensity and prolonger duration[111]. A meta-analysis by Pathirana *et al*[112] showed that breastfeeding might protect against some cardiovascular risk factors, such as Type 2 DM, in mothers with a history of gestational DM. Breastfeeding for over three months is associated with the least postpartum diabetes risk in women with gestational DM[113]. Lactation enhances glucose tolerance in mothers with gestational DM, especially in the early postpartum period. Reduced estrogen levels in breastfeeding mothers might protect against impaired glucose metabolism and consequently decrease the risk of diabetes. In addition, breastfeeding decreases the risk of obesity and further reduces the risk of type 2 DM[114].

Children born to mothers with gestational DM are more prone to prediabetes, metabolic syndrome, and obesity later in life. Gestational DM is correlated with excessive fetal growth, macrosomia, and overnutrition in utero[115]. The growth pattern in children born to women with DM (including gestational DM) is slower than controls in the first two years of life, followed by rapid weight gain and consequently increased risk of being overweight, obese, and having other metabolic disorders[116]. There is a double risk of being overweight in breastfeeders from mothers with DM compared to banked breast milk feeders at the age of two years[117]. Therefore, every effort should be made to decrease these risks. However, despite the altered breast milk composition of mothers with gestational DM, *e.g.*, reduced milk protein, there is some evidence of the beneficial effects of breastfeeding on the cardiometabolic health of their offspring. The Nurse Health study showed a significantly reduced risk of being overweight at 9-14 years in the offspring of mothers with gestational DM who breastfed for the first six months of life[118]. In addition, Ong *et al*[119] showed that breastfeeding might give some protection against undesirable fat distribution and hypertriglyceridemia in children born to mothers with gestational DM and consequently help in reducing childhood cardiometabolic risks. In the Prima Indian study, the prevalence of DM among the offspring of mothers with gestational DM was significantly lower in those with exclusive breastfeeding than those without breastfeeding at age 10-39 years after adjustment for age, sex, and birth weight[120]. Another study assessed the effects of breastfeeding and gestational DM on Hispanic children between 8 and 13 years. They found that breastfeeding protects against developing prediabetes and metabolic syndrome in the offspring with or without gestational DM[121]. However, a meta-analysis by Pathirana *et al*[112] failed to prove any protective effects of breastfeeding in IDM due to a lack of sufficient studies.

Recommendation

As breastfeeding provides adequate nutritious, easily digestible nutrients for infants, every effort should be made to encourage breastfeeding and to support the mothers to complete their mission successfully. Despite many obstacles that may enface mothers with gestational DM to start and maintain breastfeeding, every effort should be made to encourage them to breastfeed. Exclusive breastfeeding should be encouraged for 4-6 mo and complemented or supplemented for two years when possible. Information about breastfeeding, including techniques, frequency, duration, and how to overcome potential obstacles, should be available and understandable. The parents should learn and practice responsive feeding and understand the baby's cues when hungry or satisfied. The mother should also know the potential benefits of breastfeeding for her and her baby, especially when she has DM. The government should encourage and implement paid maternity leaves for at least three months to help mothers stay with their babies at home and breastfeed them. In addition, we still need to study the protective effects of breastfeeding on IDM in the short and long term. In addition, many factors are responsible for the variation of the results of the different studies, including the different methodological procedures and the differences in the target populations. Therefore, we need more extensive and multicentre studies for a longer duration and different races to ensure the beneficial roles of breastfeeding on various items of metabolic and cardiovascular health and disorders both in paediatrics and adulthood. In addition, we should request infant formula companies to do their best to mimic breast milk and reduce the gap between the advantages of breast milk and the disadvantages of infant formula. For example, these companies should revise and optimize the protein content, the amount and types of fat, and the impacts of adding probiotics, prebiotics, human milk oligosaccharides, and other well-established breast milk components.

CONCLUSION

Breastfeeding has many beneficial effects for both lactating mothers and their offspring. It protects against overweight, obesity, insulin resistance, prediabetes, DM, and metabolic syndrome in offspring regardless of gestational diabetes status. In addition, it prevents significant risk factors predisposing to cardiovascular diseases in childhood and adulthood. Therefore, every effort should be made to educate mothers about the benefits of breastfeeding for controlling DM, cardiovascular diseases, and hypertension in women and their offspring.

ARTICLE HIGHLIGHTS

Research background

Breast milk is the best and principal nutritional source for neonates. Breast milk is the best and principal nutritional source for neonates. Breast milk is the best and principal nutritional source for neonates. Breast milk is the best and principal nutritional source for neonates. Gestational diabetes has short and long-term effects on infants of diabetic mothers (IDM). Gestational diabetes has short and long-term effects on IDM.

Research motivation

Breast milk of mothers with diabetes has different compositions. Therefore, it is expected to have different effects than those from non-diabetic mothers.

Research objectives

We aimed to investigate the positive or negative cardiometabolic effects of breastfeeding on the health of IDM and their mothers.

Research methods

We searched different search engines and conducted a thorough literature review of the cardiometabolic effects of breastfeeding on the health of IDM and their mothers. We included 121 articles published in English between January, 2000 and December 15, 2022 in this review.

Research results

Most of the literature agreed that breast milk has many beneficial effects for both the mother and their infant in the short and long terms. Breastfeeding protects mothers with gestational diabetes against obesity and type 2 diabetes mellitus (DM). There is some evidence that breastfeeding has protective effects on IDM in the short and long term. However, this evidence is not strong enough due to the presence of many confounding factors and a lack of sufficient studies.

Research conclusions

Breastfeeding has numerous favorable effects for both breastfeeding mothers and their infants, protecting the offspring against overweight, obesity, insulin resistance, prediabetes, DM, and metabolic syndrome regardless of gestational diabetes status. In addition, it prevents major risk factors that predispose to cardiovascular diseases in childhood and adulthood. Every effort should be made to teach mothers the benefits of breastfeeding in controlling DM, cardiovascular diseases, and hypertension in women and their offspring.

Research perspectives

We need to study the protective effects of breastfeeding on IDM in the short and long term. We have to perform more extensive and multicentre studies for a longer duration and different races to ensure the beneficial roles of breastfeeding on various items of metabolic and cardiovascular health and disorders both in paediatrics and adulthood. We should request that those infant formula companies perform their best to mimic breast milk and reduce the gap between the advantages of breast milk and the disadvantages of infant formula.

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