World Journal of

Diabetes

World Journal of Diabetes

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Monthly Volume 14 Number 6 June 15, 2023

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ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Sanja Klobucar Majanovic, MD, PhD, Assistant Professor, Department of Endocrinology, Diabetes and Metabolic Diseases, University Hospital Rijeka, Faculty of Medicine, University of Rijeka, Rijeka 51000, Croatia. sanja.klobucarm@gmail.com

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 WJD mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

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RESPONSIBLE EDITORS FOR THIS ISSUE

State of art on the mechanisms of laparoscopic sleeve gastrectomy in treating type 2 diabetes mellitus

Fa-Shun Liu, Song Wang, Xian-Shan Guo, Zhen-Xiong Ye, Hong-Ya Zhang, Zhen Li

Fa-Shun Liu, Song Wang, Zhen-Xiong Ye, Zhen Li, Department of General Surgery, Yangpu Hospital, Tongji University School of Medicine, Shanghai 200090, China

Xian-Shan Guo, Department of Endocrinology, Xinxiang Central Hospital, Xinxiang 453000, Henan Province, China

Hong-Ya Zhang, Central Laboratory, Yangpu District Control and Prevention Center, Shanghai 200090, China

Corresponding author: Zhen Li, MD, Chief Physician, Surgeon, Department of General Surgery, Yangpu Hospital, Tongji University School of Medicine, No. 450 Tengyue Road, Yangpu District, Shanghai 200090, China. lizhen3829@126.com

Abstract

Obesity and type-2 diabetes mellitus (T2DM) are metabolic disorders. Obesity increases the risk of T2DM, and as obesity is becoming increasingly common, more individuals suffer from T2DM, which poses a considerable burden on health systems. Traditionally, pharmaceutical therapy together with lifestyle changes is used to treat obesity and T2DM to decrease the incidence of comorbidities and all-cause mortality and to increase life expectancy. Bariatric surgery is increasingly replacing other forms of treatment of morbid obesity, especially in patients with refractory obesity, owing to its many benefits including good long-term outcomes and almost no weight regain. The bariatric surgery options have markedly changed recently, and laparoscopic sleeve gastrectomy (LSG) is gradually gaining popularity. LSG has become an effective and safe treatment for type-2 diabetes and morbid obesity, with a high cost-benefit ratio. Here, we review the mechanism associated with LSG treatment of T2DM, and we discuss clinical studies and animal experiments with regard to gastrointestinal hormones, gut microbiota, bile acids, and adipokines to clarify current treatment modalities for patients with obesity and T2DM.

Key Words: Obesity; Type-2 diabetes mellitus; Laparoscopic sleeve gastrectomy; Gastrointestinal hormones; Adipokines; Gut microbiota; Bile acids

Core Tip: Obesity and type-2 diabetes mellitus (T2DM) incidence are currently increasing, and these afflictions have become important global health issues. Bariatric surgery is safe and effective for treating obesity and T2DM. The precise processes associated with this treatment, however, are somewhat unclear. Here, we review associated findings with respect to gastrointestinal hormones, intestinal microbiota, bile acids, and adipokines involved in laparoscopic sleeve gastrectomy (the most popular bariatric surgery) of T2DM patients.

INTRODUCTION

Obesity, a complicated chronic metabolic illness induced by excessive lipid accumulation, has replaced smoking as the leading cause of early mortality linked to lifestyle[1,2]. More than one-third of all nations have experienced a two-fold increase in the frequency of obesity during the 1980s, and most countries still report an increasing trend[3]. In 2015, more than 700 million adults and children were globally reported to be obese[4]. Numerous disorders, including type-2 diabetes mellitus (T2DM), afflictions of the cardiovascular system, hyperlipidemia, chronic renal disease, sleep apnea syndrome, non-alcoholic fatty liver disease (NAFLD), osteoarthritis, and metabolic syndrome, are closely associated with obesity [5].

T2DM is a prevalent metabolic condition that can damage various physiological systems and is defined by glucose metabolism problems elicited by poor insulin production and decreased insulin sensitivity[6]. The pronounced global increase in obesity, which is a major driver of T2DM, has markedly increased T2DM prevalence[7]. In 2017, more than 460 million individuals worldwide, *i.e.*, 6.28% of the global population, suffered from T2DM[8]. Obesity and T2DM have developed into important public health problems that constitute a heavy burden for the affected patients.

In addition to regular lifestyle behavior adjustments and medication, laparoscopic sleeve gastrectomy (LSG) has been acknowledged by worldwide diabetic organizations as a potent treatment of obesity and T2DM[9]. Even though the advantages of LSG for treating obesity and T2DM are commonly known, the processes by which LSG influences T2DM *via* several mechanisms, in addition to weight reduction, are still not comprehensively understood. Treatments can be optimized when the mechanisms underlying these metabolic processes and their effects on T2DM are elucidated. In this review, we focus on changes in terms of gastrointestinal hormones (GHs), adipokines, gut microbiota (GM), and bile acids (BAs) after LSG treatment of T2DM.

DEVELOPMENT OF BARIATRIC/METABOLIC SURGERY AND OVERVIEW OF PROCEDURES

Since the first bariatric surgery (BS) was performed in 1952, advances have been achieved throughout the past 70 years[10]. BS was intended to help patients lose weight and thereafter maintain normal weight; however, its importance in treating obesity-related comorbidities, particularly T2DM, has increasingly become prominent in clinical practice[11]. To improve surgery results and reduce complication rates, bariatric surgeons continually upgrade and enhance their techniques, and current bariatric operations include vertical-banded gastroplasty, duodenal switch, jejunoileal bypass, biliopancreatic diversion, adjustable gastric banding, Roux-en-Y gastric bypass (RYGB), and sleeve gastrectomy (SG)[12]. Additionally, BS is mostly carried out through laparoscopy due to the advances of lumpectomy surgery.

The most frequently performed BS techniques are RYGB and SG[13]. The first variant of SG was described by Marceau *et al*[14] in 1993; it is a more physiologic variation of gastroplasty, which is normally a restrictive treatment using a longer, less curved vertical gastric tube to reduce stomach capacity. Despite their anatomical distinctions, both treatments have been proven safe and effective for treating obesity and T2DM[15]. BS can markedly decrease all-cause mortality and enhance life expectancy in obese adult patients, compared to standard obesity therapy, as evidenced by long-term follow-up of a large sample population. In addition, individuals who are overweight and suffer from T2DM benefit more from this treatment than those who suffer from obesity only[16]. A long-term follow-up study of 146 patients approaching 10 years showed complete remission of T2DM after LSG in 72.2%, significant improvement in 25.1%, and no change in only 2.7%[17]. The treatment effect of LSG

on T2DM in morbidly obese patients was the same compared to laparoscopic RYGB (LRYGB), as demonstrated by a meta-analysis containing 9 studies, in which the remission rates of T2DM were 82.3% and 80.7% for LRYGB and LSG, respectively[18]. In addition, a meta-analysis containing 33 studies with 4109 patients showed that patients receiving LSG experienced more significant improvement or remission of diabetes than those receiving laparoscopic adjustable gastric banding (LAGB)[19]. A meta-analysis designed for 1108 adult subjects showed that the probability of T2DM mitigation after LSG was 61.4%, significantly higher than in the medication group (2.5%). Based on the above findings, the remission rate of T2DM after LSG was not significantly different from LRYGB but significantly higher than drug treatment and LAGB[20].

Surgeons performing BS and patients tend to choose LSG over other BS because of its lower risk of complications, compared to other surgical procedures; further, it is less invasive, preserves the body's original natural channels, and has better clinical outcomes. Currently, LSG is globally the most common BS[21]. Between 2010 and 2018, the proportion of LSG among BS techniques increased from 2% to 61%, whereas that of RYGB decreased from 55% to 17%[22]. According to the International Federation for Surgery of Obesity Global Registry, 833678 weight-reduction procedures were recorded globally in 2019; however, only 1% of individuals qualified for surgical reasons received surgical treatment[23,24]. Thus, there is considerable room for expansion of bariatric metabolic surgery. Considering the advances in BS options, we focus on the mechanisms of LSG relieving T2DM. The remission rate of T2DM after SG is approximately 65%[25], and this process involves, for example, GHs, GM, BAs, adipokines, the nervous system, and other potential mechanisms that are addressed here.

GASTROINTESTINAL HORMONES

Ghrelin

Ghrelin, also referred to as the "hunger hormone", is a peptide of 28 amino acids predominantly generated by gastric fundus X/A cells. During fasting, ghrelin expression increases, and it is reduced after eating[26]. Ghrelin regulates the energy balance, increases the sensation of hunger, stimulates growth hormone release from the hypothalamus and anterior pituitary, and stimulates food intake to facilitate the buildup of adipose tissue[27,28]. Additionally, ghrelin increases muscle insulin resistance (IR) and controls peripheral glucose homeostasis by lowering glucose-stimulated insulin release[29,30]. In extremely obese individuals, ghrelin prevents the appropriate inhibitory response to food intake and does not return to normal after losing weight without surgery[31,32]. Kalinowski *et al*[33] found that glucose metabolism improved in obese patients with BS, with reduced ghrelin levels after LSG and increased levels after RYGB. The same outcomes were obtained in other long-term follow-up trials, with patients reporting a significant decrease in ghrelin levels after LSG[34]. Stoica *et al*[35] confirmed this finding in a study on Wistar rats showing that LSG markedly decreased the levels of circulating acylated ghrelin. The primary location of ghrelin production is removed through LSG, which may be the primary cause of reduced ghrelin levels post-surgery. This ghrelin decrease after LSG likely explains the subsequent glycemic improvement as ghrelin is associated with higher circulating insulin and glucagon levels[36]. However, in a study on ghrelin-deficient and wild-type mice, the responses to LSG resembled those after glycemic control, which implies that ghrelin may not be required to improve the glucose metabolism[37]. The studies cited above concluded that LSG substantially affects ghrelin production but that this effect was not the single causative factor of postoperative T2DM remission.

Peptide tyrosine tyrosine

As a member of the pancreatic polypeptide-fold family, peptide tyrosine tyrosine (PYY) is a digestive hormone released after eating by the L-cells among intestinal endocrine cells of the distal ileum and colonic mucosa, and in rodents, it is considered a satiety signal[38]. PYY may also affect insulin sensitivity and glucose absorption by acting on Y2 receptors, and it may modulate insulin secretion by acting on islets[39]. Reduced PYY levels occur in obese people during fasting and after eating, possibly because PYY synthesis, release, or clearance is impeded[39]. Exogenous PYY has recently attracted attention as an anti-obesity agent that can reduce food intake, delay stomach emptying, and lower the glycemic index[40,41]. Potential LSG-induced alterations of PYY levels are currently controversial. One prominent question is whether PYY levels change after LSG surgery. Most studies concluded that PYY is elevated due to LSG[42-44], whereas one study suggested that PYY secretion, although numerically increased, is not statistically different from baseline[45]; however, considering the small number of patients included in this study (only six cases), this may not be a general pattern. The other question is whether increased PYY is restored to its baseline levels within a certain period after LSG.

Arakawa *et al*[41] observed an increase in PYY 26 wk after surgery but not after 52 wk. Similar results were obtained in a different study, showing higher PYY levels immediately after surgery, which then decreased to baseline levels within one year[44]; PYY secretion did, however, continue to increase postoperatively and remained above baseline levels at 18 mo, according to Alamuddin *et al*[42]. In an animal study, non-obese diabetic Goto-Kakizaki (GK) rats that were subjected to LSG showed substantial improvements in glycemic control, a significant decrease in glycated hemoglobin, and an

increase in diet-induced PYY[46]. Moreover, in diet- and streptozocin (STZ)-induced diabetic obese mice, LSG can increase PYY levels. Animals subjected to surgery also show higher glucose tolerance and fasting insulin improvement, and their insulin secretion increases and peaks faster following glucose infusion[47]. Boza *et al*[47] additionally performed ileal transposition with LSG, and compound surgery resulted in a considerable reduction in food intake, increased PYY levels, and improved glucose tolerance in obese diabetic mice. Current research suggests that PYY levels are increased in mice and humans subjected to LSG, which is directly related to lower food consumption. Further fundamental research is required to determine whether a direct connection exists between higher PYY and better insulin release and glucose tolerance.

Oxyntomodulin

Oxyntomodulin (OXM), like PYY, is produced by intestinal L cells. It participates in the control of satiety, influences the production of hydrochloric acid by gastric secretion glands, and exerts a biological activity similar to that of glucagon[48,49]. OXM has not yet been linked to a particular receptor, but intriguingly, it affects glucagon-like peptide (GLP)-1 receptors in the hypothalamic arcuate nucleus[50]. Furthermore, it exhibits entero-insulinotropic effects and β cell-protecting qualities[51]. According to previous studies, OXM may boost energy expenditure and control blood glucose levels in obese people while suppressing appetite and reducing food intake[52,53]. In obese individuals with T2DM, OXM combined with GLP-1 and PYY has been demonstrated to improve glycemia and body weight[54]. Few studies examined how BS affects OXM, particularly when the surgical strategy is restricted to LSG; thus, little is known about changes in OXM following LSG. Nielsen *et al*[55] reported that post-LSG patients exhibited increased OXM production, which was correlated with body weight and postoperative dietary preferences. After RYGB, weight reduction may be predicted by early postprandial OXM, according to a different study[56]. Laferrère *et al*[57] conducted oral glucose tolerance trials and found that peak OXM levels were considerably higher in the surgery group compared to the control diet group and corresponded with an increase in PYY. Further, OXM levels following RYGB surgery did not change while fasting. In mice, exogenous OXM increases glucose-induced insulin secretion, energy expenditure, and weight loss[58]. This effect of OXM may be due to its impact on the GLP-1 receptor (GLP-1R) as it does not stimulate insulin secretion in GLP-1R-/- mice[59]. The effect of exogenous OXM on T2DM has been partly established, however, further research is needed to understand how it is affected by LSG and other types of BS. Intriguingly, two studies have revealed that OXM might be a predictor of weight reduction after BS. We hypothesize that this impact may be associated with changes in dietary practice and satiety.

Cholecystokinin

Cholecystokinin (CCK) was first described in 1982[60], and as suggested by its designation, it is a peptide hormone which can cause gallbladder contraction linked to the gastrointestinal system. According to recent studies, CKK receptors are expressed in the pancreas, central nervous system, gallbladder smooth muscle, and stomach mucosa[61]. CCK interacts with CCK-1 receptors in distinct areas of the hindbrain to signal satiety and decrease food intake[62]. CCK has also been linked to neurophysiological processes, including anxiety, sadness, pain, learning, and memory[63,64]. It controls stomach acid production, reduces BA release, and impacts gastrointestinal motility in the gut[65,66]. In aged mice, CCK expression in β cells increases the area of the pancreas and shields the cells from STZ-induced diabetes and apoptosis, demonstrating a protective impact on β cells[62]. Frequent ravenous hunger of obese patients may be explained by the fact that insensitivity of vagal afferent neurons to CCK is decreased which reduces the drug's impact on satiety[60]. CCK and associated peptide hormones can successfully be used as adjuvant therapy for treating T2DM and obesity[67]. In high-fat diet (HFD) mice, CCK analogs can lower caloric intake, reduce body weight, and increase insulin sensitivity[68]. Numerous studies have shown that LSG significantly affects the levels of circulating CCK, thus improving glucose homeostasis and improving homeostasis model assessment of IR (HOMA-IR)[69,43]. Additionally, elevated CCK appears to inhibit sympathetic action and subsequently inhibits the intrarenal renin-angiotensin system, producing a hypotensive effect[70]. LSG has a stronger CCK-increasing effect than RYGB; however, it seems to be associated with lower remission rates in T2DM patients[71]. According to current research, CCK has a favorable function in preserving glucose homeostasis in T2DM, and one potential explanation may be its protective effects on pancreatic β cells. In cases with obesity, the weight-reduction effect of CCK may be mediated by a response of the central nervous system that re-establishes normal satiety signaling and reduces food ingestion. However, as there is no clear correlation between the increase in CCK and frequency of remission of T2DM after BS, it is not entirely conclusive to explain T2DM by changes in it alone.

GLP-1

GLP-1 is considered the most "successful" peptide hormone currently available. It is predominantly produced by intestinal L cells, and is a fundamental compound of several T2DM and obesity medications and of novel medications currently under research[72]. Under physiological circumstances, ingested food (including carbohydrates, glucose, proteins, and BAs) stimulates L cells scattered

throughout the epithelium to release GLP-1 into the blood at a rate corresponding to food absorption [73]. This hormone is important in coordinating postprandial glucose homeostasis. GLP-1 stimulates the release of postprandial insulin, and activation of GLP-1R in pancreatic β cells stimulates the release of insulin, which depends on plasma glucose levels[74]. When β cells perceive elevated plasma glucose levels and GLP-1 signals from the intestine, it enhances insulin release after glucose intake, which is also known as the intestinal proinsulin effect[75]. Meanwhile, GLP-1 prevents pancreatic α cells from releasing glucagon[76], and it regulates gastric emptying, thus influencing appetite and contributing to a sensation of satiety. GLP-1 contributes to the ileal brake, allowing nutrients to enter the duodenum at the same rate as absorbed in the small intestine[77]. By targeting GLP-1R in the brainstem or hypothalamus, GLP-1 decreases hunger and increases satiety, which is complementary to the effects of PYY; however, both originate from L cells[78,79]. In T2DM, GLP-1 secretion is reduced, and the effect of entero-insulin is diminished[80]. However, this may be a consequence of T2DM rather than an etiology because non-T2DM patients with elevated blood glucose show a marked decrease in GLP-1 Levels[81]. The study of Shehata *et al*[82] showed that in obese adolescents with T2DM, LSG significantly increased GLP-1 Levels in the early postoperative period (until six months after surgery). However, it did not produce the same effect during the late postoperative period (12 mo after surgery). Furthermore, the size of the antrum was not linked to higher GLP-1, better glucose control, or less IR, but to higher T2DM remission rates. Min *et al*[83] came to similar conclusions, as GLP-1 Levels were increased in the early stage after surgery, but this effect was not persistent. Significant reductions in glycosylated hemoglobin (HbA1c) and IR predict improvement of T2DM. Vigneshwaran *et al*[84] also found that LSG led to increased GLP-1 Levels six months after surgery in T2DM patients who were not morbidly obese, but they did not record GLP-1 Levels thereafter. Further, obese people without T2DM also showed low insulin sensitivity and high insulin levels in the blood, compared to healthy controls. After LSG intervention. patients showed higher insulin sensitivity and markedly higher GLP-1 Levels[85].

In contrast, Rigamonti *et al*[86] compared GLP-1 Levels before and after surgery and examined how food ingestion rates affected GLP-1 secretion. They found no significant difference in GLP-1 Levels, but they proposed that LSG would make patients less resistant to insulin. However, who underwent RYGB showed higher GLP-1 Levels, better β cell function, and a higher chance of remission from T2DM[87]. In an animal study, Garibay *et al*[88] showed that SG helps better control glucose levels by improving β cell GLP-1R signaling and increasing glucose-stimulated insulin secretion. Li *et al*[89] suggested that improved glucose metabolism in GK rats with SG was caused by increased GLP-1 secretion, which was achieved by increasing the amount of GLP-1 in the plasma through increasing GLP-1 production in the jejunal and ileal mucosa.

Nevertheless, other studies suggest a different perspective. Wilson-Pérez *et al*[90] used GLP-1R-deficient mice which after SG did not differ significantly from wild-type controls in terms of weight and body fat reduction, improved glucose tolerance, food intake, and food preference. The authors concluded that GLP-1R activity was not required for SG to improve glucose metabolism and reduce body weight. Evidence from recent studies supports the notion that GLP-1 is crucial for maintaining glucose homeostasis, and the prospect of developing effective treatments is encouraging. As a hormone with an intestinal proinsulin effect, production of GLP-1 may be decreased during T2DM. The effect of LSG on GLP-1 currently prefers the ability of LSG to increase GLP-1 Levels in the early postoperative period. It may alter glucose homeostasis and help cure T2DM by boosting intestinal L-cell GLP-1 production and promoting GLP-1 signaling in pancreatic β cells. However, it remains controversial why SG produces the same surgical effect in mice, even without GLP-1R. Therefore, further studies are required to determine how GLP-1 influences glucose metabolism in T2DM after LSG.

GLP-2
GLP-2 consists of 33 amino acids and is encoded at the carboxyl terminus of the GLP-1 sequence in the glucagon gene. Like GLP-1, it is predominantly produced by enteroendocrine L cells in the ileum and large intestine[91]. It is produced in response to food stimulation in the gut, and GLP-2 is primarily responsible for inhibiting gastrointestinal motility and intestinal nutrition (enhancement of intestinal growth, digestion, absorption, barrier function, and blood flow)[92]. Due to its distinct intestinal nutrition effects, the use of GLP-2 analogs for the treatment of intestinal failure can markedly reduce the frequency of required parenteral nourishment[93]. GLP-2 contributes to preserving the energy balance, and in particular, it promotes nutritional absorption in the gastrointestinal system; this is achieved not only by enterotropic action but also by decelerating gastrointestinal motility, which extend the duration of nutrient digestion and absorption. Intriguingly, GLP-2 is a peptide hormone that has been associated with anorexia[94]. Its receptor, GLP-2R, is expressed in the brainstem, hippocampus, and hypothalamus, which are thought to be essential for maintaining homeostasis of energy[95]. Peripheral GLP-2 injection decreases food intake in mice on the short term[96].

Furthermore, mice with a specific GLP-2R deficiency in proopiomelanocortin neurons show increased plasma insulin and hepatic glucose production as well as glucose intolerance[97]. Moreover, endogenous GLP-2 demonstrated a protective effect against IR in HFD mice[98]. Romero *et al*[99] observed an increase in GLP-2 Levels and an improvement in glucose tolerance in the first postoperative phase after LSG. Cummings *et al*[100] attained similar outcomes in an animal experiment, where SG enhanced glucolipid metabolism and postponed the development of diabetes in University of California Davis

(UCD)-T2DM rats, in addition to increasing GLP-2 Levels. GLP-2 regulates the circulating BAs, although Patel *et al*[101] showed that it is not required for body weight and glucose homeostasis in GLP-2 receptor-deficient SG mice. However, Patel *et al*[101] also found that GLP-2 regulates circulating BAs, but it is not required for body weight and glucose homeostasis in GLP-2R-deficient SG mice. In conclusion, in-depth research on GLP-2 is lacking, and data to determine how LSG affects GLP-2, particularly in humans, are currently insufficient. The available data merely provide evidence for the hypothesis that the observed increase in GLP-2 Levels after LSG is likely to play several functions in homeostatic processes *in vivo*, whereas the precise mechanisms remain unknown.

Glucose-dependent insulinotropic polypeptide

Following food ingestion, endocrine K cells in the crypt-villi axis produce glucose-dependent insulinotropic polypeptide (GIP), a protein comprising 42 amino acids. This hormone was originally designated gastric inhibitory polypeptide because of its capacity to reduce stomach secretion and motility[102]. However, GIP was then identified as an incretin hormone capable of enhancing glucose-dependent insulin secretion from pancreatic β cells and thus received its current designation[103]. GIP exerts two functions. As a sister hormone of GLP-1, GIP exerts the same proinsulin action, and the loss of effects of entero-functional insulin is the primary cause of poor postprandial glycemic control in T2DM[104]. GIP agonists have been developed for the treatment of T2DM and obesity[105]; however, it is crucial to note that GIP agonists do not effectively reduce blood sugar levels in T2DM; nevertheless, when coupled with GLP-1 and GIP agonists, their benefits are significantly larger than those of GLP-1 alone[106]. GIP, by contrast, may influence the distribution of fat in adipose and non-adipose tissues, causing ectopic fat deposition and stimulating the accumulation of visceral and hepatic fat[107]. The major source of circulating non-esterified fatty acids is visceral fat, and a persistent increase in these acids is linked to the development of IR and T2DM[108]. Additionally, inflammation of pro-inflammatory adipokines and adipose tissue may be exacerbated by GIP[109]. Excessive GIP production contribute to the development of fatty liver and NAFLD[110]. GIP receptor antagonists may restore obesity, IR, and related metabolic problems in mice caused by prolonged HFD intake, thus they are also a viable treatment option[111]. According to one study, GIP level of patients increased linearly following LSG and continued to increase for four years, resulting in better glycemic management[83]. A study by Romero *et al*[99] on extremely obese individuals revealed an elevated GIP response following LSG, whereas after RYGB, no comparable reaction was observed. Other results suggest that RYGB reduces postprandial GIP secretion, owing to restricted food transit through the duodenum and jejunum[112]. In STZ-induced diabetic mice, Wang *et al*[113] found no change in GIP between SG- and sham-operated groups, and SG had no mitigating impact on STZ-induced diabetes. GIP seems to exert contrary functions in obese T2DM patients. However, this hormone belongs to the enterotrophic insulin family, and its agonists may be utilized to treat T2DM and obesity, resulting in hypoglycemia and weight reduction benefits. By contrast, it has been shown to enhance adipose inflammation, induce fat deposition, and to be linked to the onset of fatty liver and NAFLD. With regard to how BS may affect GIP, LSG seems to raise GIP levels, whereas RYGB causes a decrease in GIP production, depending on the surgical method. Given that GIP exerts contrasting functions, currently available studies cannot conclusively determine whether changes in GIP secretion after LSG are advantageous or harmful.

Gastrin

Gastrin is produced in the G cells of the gastric sinus and duodenum, and it is released in response to stimulation by the vagus nerve and gastrin-releasing peptide[114]. This hormone family comprises numerous peptides, with varying levels of biological activity and lengths[115]. The primary roles of gastrin include inducing gastric acid production in the stomach *via* a Ca-dependent release mechanism, acting on intestinal chromophobic cells in the fundus to trigger histamine release, stimulating the development and motility of the gastric mucosa, and suppressing hunger[116]. Recent studies focused on the relationship between gastrin and the onset and progression of gastrointestinal cancers, particularly neuroendocrine tumors[117]. IR and abdominal obesity are correlated with low gastrin levels [118]. Gastrin and GLP-1 dual agonists exert immunomodulatory effects that enhance insulin levels and β-cell mass in non-obese diabetic mice, eventually improving glycemic control. Furthermore, in individuals with T2DM, the addition of proton pump inhibitors (PPI) to glucose-lowering medications markedly raised gastrin levels, enhanced β cell activity, and reduced HbA1c levels[119-121]. A trend towards increased gastrin secretion after SG was observed in female patients who had undergone BS compared to patients receiving a protein-rich meal mix. However, no statistically significant difference was observed, while gastrin was significantly lower after RYGB. Notably, a negative correlation occurred between gastrin secretion and glucose levels after SG[118]. Grong *et al*[122] found that SG had superior effects in inducing hypergastrinemia, lowering HbA1c, and improving glycemic control in a GK rat model. In a subsequent study, the authors assessed the -cell mass in GK rats using three-dimensional optical projection tomography, showing that -cell mass was maximally preserved after SG, which may be related to high gastrin levels and long-term improvement in glycemic parameters following surgery[123]. Grong *et al*[124] also suggested the presence of circulating high gastrin in GK rats after SG. However, this was similar to the result after PPI intervention, with no difference in glycemic control between the two groups, and SG did not improve β cell mass. Few human studies on

gastrin changes after SG are available, and current evidence suggests the presence of high gastrin levels after SG, which may have a positive effect on glycemic control in T2DM; however, the precise mechanisms involved are unclear. In general, the results are inconsistent as to whether high gastrin improves β cell quality.

Fibroblast growth factor 19 and fibroblast growth factor 21

At least 22 protein family members of fibroblast growth factors (FGFs) are associated with angiogenesis, wound healing, metabolic control, and cell growth, development, and migration differentiation[125]. The majority of these work as paracrine or autocrine factors. FGF19, FGF21, and FGF23 are hormone-like members of the FGF family and have certain structural characteristics that facilitate endocrine effects[126]. FGF19 is produced in the brain, gallbladder, and distal small intestine. It inhibits hunger and regulates BA and nutrition metabolism, glucose and lipid metabolism, energy expenditure, and obesity[127]. FGF21 controls lipid and carbohydrate metabolism, elicits white adipose tissue (WAT) thermogenesis and browning, indirectly increases insulin synthesis in the pancreas, improves insulin sensitivity, and decreases food intake[128]. FGF23 is a hormone produced by osteoblasts and osteoclasts in the skeleton and is primarily involved in mineral metabolism to control phosphate levels[129]. According to several studies, there is a significant increase in FGF19 following SG, and this increase is linked to better glycemic control and reduced systemic inflammation[130-132]. Yang *et al*[133] observed an increase in FGF19 in VSG but no changes in RYGB. A meta-analysis revealed an increase in FGF19 and a negative correlation between FGF19 and BMI after SG[134]. Huang *et al*[135] noted that higher FGF19 Levels and reduced BA levels after SG may play a role in T2DM remission and NAFLD improvement; they also hypothesized that low preoperative FGF 19 Levels may predict improvement of NAFLD.

With respect to FGF21, Khan *et al*[136] found a link between elevated FGF21 and weight loss after SG, indicating that FGF21 may play a part in the postoperative energy balance. By contrast, Nielsen *et al* [137] did not detect changes in FGF21 after SG, and FGF21 Levels were not related with food choice. FGF19 Levels were decreased and FGF21 Levels were increased in obese patients, and FGF21 Levels further increased when obese patients showed T2DM. SG increased FGF19 Levels while decreasing the unnaturally increased FGF21 Levels. The authors concluded that FGF19 Levels were mostly related to physical obesity, particularly visceral obesity, whereas those of FGF21 were primarily linked to glucose homeostasis[138]. Yen *et al*[139] confirmed this and further observed a substantial decrease in FGF21 Levels after SG and a strong positive association between FGF21 and C-peptide, insulin, and the homeostasis model evaluation of the postoperative IR index.

In conclusion, the available studies are in line with our findings that FGF19 is typically elevated in the postoperative period and that it may control the release of BAs to produce its effects. The elevation of FGF19 after SG is not specifically correlated with T2DM but is linked to a decrease in the body weight index. Contrarily, FGF21, which is frequently increased in obese patients with T2DM, has an independent function in obesity and is linked to metabolic syndrome, hyperinsulinemia, onset of diabetes, aberrant glucose metabolism, and IR[140]. Due to its potential to ameliorate the FGF21 increase induced by obesity or T2DM, SG may play a significant part in preserving glucose homeostasis. FGF21 should be further studied, and it may be a more important metabolic marker of illness in T2DM than FGF19.

Overall, the control of different components of the gut-brain axis, the gut-adipose tissue axis, the gut-liver axis, the gut-pancreatic axis, and the gut-muscle axis all play a role in the overall complexity of the gastrointestinal hormonal alterations after LSG. The surgical method used in RYGB (partial removal of the small intestine and stomach) may explain endocrine differences between LSG and RYGB; this also suggests that the two treatments affect T2DM differently because of such discrepancies. Although the benefits and drawbacks of the two approaches are not entirely clear, one may infer from the few available data that the potential of LSG ability to relieve T2DM is connected to GHs, which may result from systemic rather than specific hormonal alterations.

ADIPOKINES

Adipose tissue is divided into WAT and brown adipose tissue (BAT), classically considered a long-term storage organ that releases free fatty acids to meet the body's energy requirements during fasting or thermoregulation and has a mechanical protective impact on internal organs[141,142]. According to current studies, adipose tissue is one of the major endocrine organs in the body and plays a significant role in systemic homeostasis[143]. Adipocytes are metabolically active, and they are effective secretory cells that can release large quantities of adipokines. Adipokines may influence several biological processes, including appetite regulation, inflammatory and immune functions, glucose and lipid metabolism, cardiovascular homeostasis and reproduction, and other essential physiological processes [144]. This review focuses on T2DM and obesity; hence, other physiological functions will not be described in any great detail. Leptin, adiponectin, resistin, and vaspin are adipokines associated with glucose metabolism. Insulin sensitivity is linked to leptin, adiponectin, chemerin, and omentin, whereas

IR is associated with apeline and nesfatin-1. By contrast, leptin and vaspin are also important in controlling appetite[145,146]. As a result, T2DM and adipokine changes are tightly associated in obese people. Below, we provide more details on how LSG affects specific adipokine metabolism processes and its potential impact on T2DM and also summarize the approximate mechanism in Figure 1.

Leptin

Leptin has a tertiary structure of a globular protein, comprising 167 amino acids. It is predominantly synthesized in white adipose tissue, and primarily acts on trans-modal receptors to exert its effects[147]. Food consumption, systemic adiposity, and hormones affect the amount of leptin that is secreted, with insulin playing a significant regulatory role[148]. Prolonged hyperinsulinemia leads to an increase in circulating leptin concentration[149]. Considering the IR status of obese patients, high leptin levels are likewise a characteristic of obesity. Leptin thus controls hunger, satiety, food intake, and energy use [150].

Meanwhile, it may play an insulin-sensitizing role and is an important regulator of β cell mass and survival. Recombinant leptin has been established for obesity treatment based on its various important physiological roles. However, little progress has been made, which may be due to long-term leptin-resistance during obesity[151]. When such resistance is reduced, recombinant leptin treatment produces effective weight reduction and glycemic control[152]. Thus, studying the alterations in leptin that occur after LSG and how they affect T2DM and obesity is crucial. Numerous studies have produced similar findings, and the impact of LSG on leptin is generally beneficial, with a discernible decrease in leptin levels after surgery that remained throughout long-term follow-up[33,34,153]. Mazahreh *et al*[154] concluded that LSG increased the expression level of leptin receptors, which alleviated leptin resistance. Leptin levels and IR were correlated in patients, and pre-LSG leptin levels were predictive of IR, according to Hany *et al*[155]. Additionally, Arble *et al*[156] also reported that SG improves ventilatory drive in patients with sleep apnea through a leptin-dependent mechanism. Stoica *et al*[35] showed that SG decreased leptin expression in mice. Similarly, Du *et al*[157] discovered that SG lowered leptin expression in HFD-fed mice, which caused translocation of glucose transporter protein 2; resulting in inhibition of intestinal glucose absorption. In leptin receptor-knockout mice, long-term weight reduction following SG was shown to require the action of leptin; however, the improvement in blood glucose does not seem to depend on leptin. The authors concluded that a significant improvement in blood glucose caused by SG through enhanced insulin sensitivity, independent of reduced feeding and weight loss[158]. LSG has a well-documented impact on lowering circulating leptin levels and enhancing leptin resistance, and these beneficial effects have been linked to several healthful physiological processes. However, it remains controversial whether changes in leptin levels have beneficial effects on glucose metabolism in T2DM, which may be involved partly by reducing glucose uptake and improving IR, among other effects. The role of leptin in this process is not all or nothing, but good or better.

Adiponectin

WAT secretes adiponectin, one of the most prevalent adipokines in the bloodstream of humans[159]. As a secreted protein, it functions by interacting with the cell membrane receptors adiponectin receptor (AdipoR) 1 and AdipoR2. AdipoR1 is primarily expressed in liver and skeletal muscle tissue, and AdipoR2 is predominantly expressed in the liver[160]. Adiponectin increases skeletal muscle glucose absorption and fatty acid oxidation, thus inhibiting gluconeogenesis in the liver[161,162]. Additionally, adiponectin has anti-diabetic properties and activates the AMP-activated protein kinase (AMPK) pathway, which interacts with the AdipoR1 receptor to elicit insulin sensitization[163]. Furthermore, lipocalin exerts anti-inflammatory effects, it is linked to the onset of atherosclerosis, and it effectively inhibits the activation of the nuclear transcription factor-kappa B (NF-kB) pathway and production of the NF-kB nuclear protein p65[164]. Obese patients with T2DM exhibit reduced adiponectin levels which are associated with increased expression of pro-inflammatory cytokines; this may also be associated with low-grade chronic inflammation[165]. According to previous studies, increasing the amount of lipocalin in the blood would be a viable therapeutic approach to treat disorders caused by obesity. Thiazolidinediones, which act as peroxisome proliferator-activated receptor γ (PPAR-γ) agonists, may raise adiponectin levels and successfully regulate blood sugar. However, their applicability is more constrained owing to lower safety (with adverse side effect including hepatotoxicity, heart failure, edema, and reduced bone density)[166]. Lopez-Nava *et al*[167] reported increased adiponectin levels after LSG, no equivalent changes were seen after endoscopic SG, and patients exhibited increased weight loss following LSG. Rafey *et al*[168] obtained similar results with increased circulating adiponectin after LSG, and the authors suggested that the leptin-to-adiponectin ratio was correlated with improved insulin sensitivity and weight loss, and that this ratio decreased significantly after surgery. Šebunova *et al*[169] took an identical perspective: Adiponectin levels increased after BS, however, the authors did not distinguish between various surgical techniques. In GK rats, SG increased serum adiponectin and adipose tissue PPAR-γ expression, decreased IR, and enhanced adipose tissue health and angiogenesis[170]. Adiponectin may have a role in improving glucolipid metabolism and delaying the development of T2DM in UCD-T2DM mice when SG is performed[100]. In addition, a combination of SG and partial small bowel resection resulted in elevated adiponectin levels, which may contribute to improved glucose homeostasis[171]. Adiponectin exerts a significant role in glucose
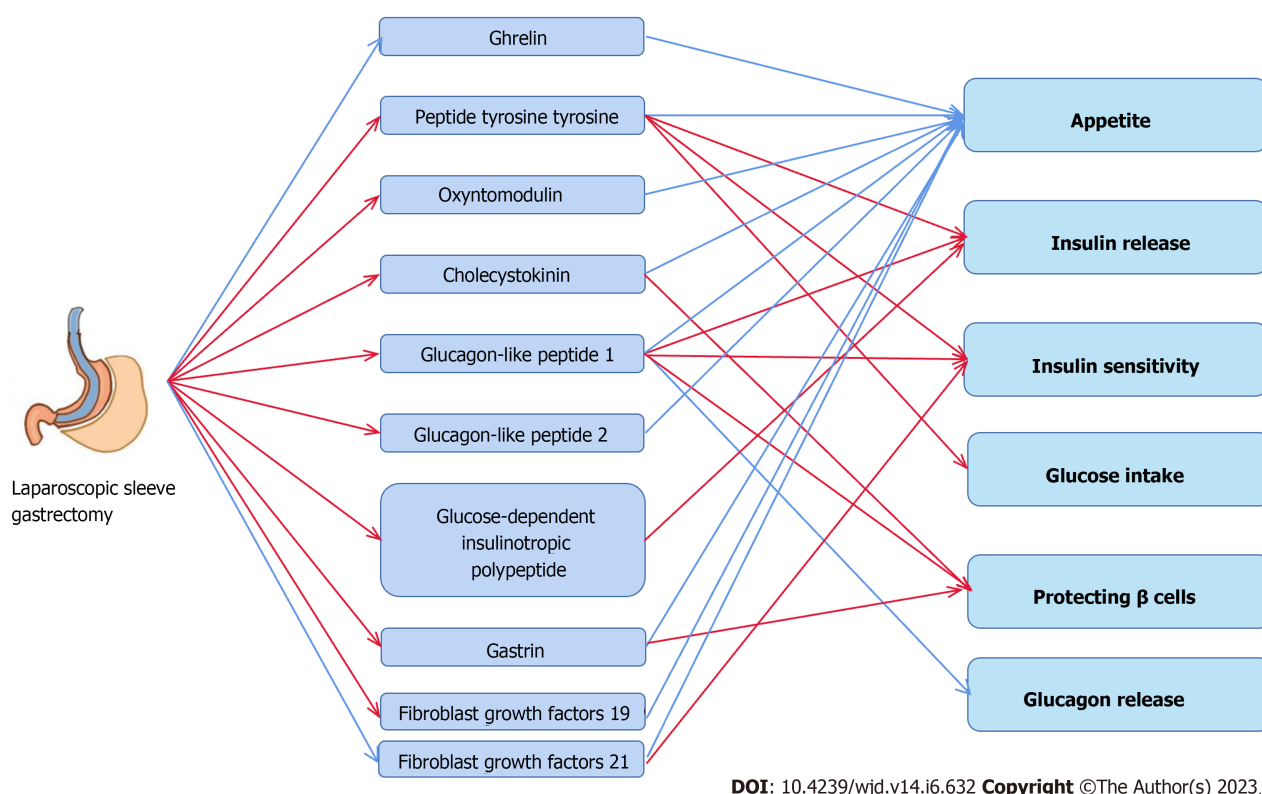
Figure 1 Mechanism of laparoscopic sleeve gastrectomy to improve type 2 diabetes mellitus through gastrointestinal hormones. Red arrows represent facilitation, while blue arrows represent inhibition.

metabolism, whether in patients with T2DM, obesity, or both. Elevating the circulating adiponectin levels through medication seems to be an effective option; however, this treatment modality should be considered with caution regarding the aspect of safety. The effect of LSG on adiponectin is currently presumed consistent, with a postoperative increase, which may be one of the mechanisms by which LSG can help treat T2DM and obesity. Risks and safety of LSG are manageable for specialist weight loss metabolic surgeons, which is one of its advantages over established pharmacological approaches.

Apelin
Apelin is a late-discovered adipokine peptide with multiple active isoforms. Its receptor, apelin-angiotensin receptor-like (APJ), is an extensively distributed G protein-coupled receptor[172]. Various tissues and cells in the human body contain apelin/APJ, which perform various physiological tasks, including controlling food intake, cell proliferation, and angiogenesis[173]. Apelin is recognized as a helpful adipokine and, like adiponectin, is thought to be an insulin sensitizer[174]. Exogenous apelin supplementation is still beneficial for IR and for the glucose metabolism, even when endogenous apelin levels are high in obese patients and those with T2DM[175]. Exogenous apelin has been shown to improve insulinotropic activity, adipocyte glucose absorption, and insulin release in obese mice, and it is similarly beneficial in human patients[176,177]. Soriguer *et al*[175] reported a significant decrease in apelin levels in morbidly obese patients with impaired fasting glucose or T2DM due to BS. Apelin levels were significantly positively correlated with changes in serum glucose and negatively correlated with insulin sensitivity. Arica *et al*[178] observed that laparoscopic gastric banding reduced elevated apelin levels in obese morbidly obese patients. However, we were unable to identify studies on the effects of LSG on apelin. As a novel therapeutic target and important biomarker for metabolic illnesses, including diabetes and obesity, the apelin/APJ signaling pathway has recently attracted attention. However, few studies on apelin and BS are available, and they suggest that apelin levels decrease postoperatively, which seems to be disadvantageous.

Nesfatin-1
The novel adipokine nesfatin-1 is not only released by adipose tissue, but its synthesis and secretion have also been observed in central nervous tissues including the hypothalamus[179]. So far, the nesfatin-1 receptor remains unknown; however, specific binding sites have been found in the central nervous system, gastrointestinal tract, and pancreas[180]. Nesfatin-1 is considered an efficient anorexigenic peptide with regulatory effects on energy metabolism through reducing food intake[181]. Nesfatin-1 expression is lower in obese people, and its levels are negatively correlated with body mass

index, weight, and adiposity[182]. Similar observations were made in T2DM patients, whose nesfatin-1 Levels were lower than those of healthy subjects or T1DM patients[183]. Nesfatin-1 stimulates insulin secretion, increases proinsulinogen mRNA expression, and has antihyperglycemic effects during glucose metabolism[184]. A previous study showed that supplementation with exogenous nesfatin-1 elicited resistance to hyperglycemia in mice, suggesting that nesfatin-1 may be a potential therapeutic target for T2DM[185]. According to several studies, LSG raises postoperative nesfatin-1 Levels in patients. Nesfatin-1 has been linked to a reduction in postoperative appetite, according to Dogan *et al* [186], whereas Yang *et al*[187] observed a link between nesfatin-1 and NAFLD. Lee *et al*[188] demonstrated that nesfatin-1 decreased after SG or RYGB, and they proposed a link between nesfatin-1 and glycemic control.

In contrast, Majorczyk *et al*[189] came to the exact opposite conclusion, suggesting that LSG decreases nesfatin-1 Levels and that there is no significant correlation between nesfatin-1 and improvement in body weight or glucose metabolism. There is a controversy with regard to LSG's impact on nesfatin-1, with starkly contrasting opinions. The correlation between nesfatin-1 and weight, appetite, and hepatic steatosis after LSG has been demonstrated, however, only one study has shown a correlation between nesfatin-1 and glycemic control after LSG. Thus, nesfatin-1 may play a minor role in the LSG-mediated remission of T2DM.

Resistin

Resistin is a specific adipokine specifically expressed and secreted by adipose tissue[190]. Its effects involve endocrine, autocrine, and paracrine mechanisms, however, its receptor is unknown[191]. Resistin is considered a connection between obesity and T2DM as it reportedly opposes the action of insulin and interferes with glucose homeostasis *in vivo*, which results in the progress of T2DM[192]. Resistin is also a pro-inflammatory regulator of macrophages, peripheral blood mononuclear cells, and vascular cells, with pro-inflammatory actions and higher expression during pathological states of inflammation, according to recent studies[193,194]. Resistin levels were positively correlated with IR in T2DM patients with hyperresistinemia and in obese people, according to a meta-analysis of 20 studies. However, no such association was found in patients with normal resistin levels[195]. A study showed that leptin and resistin levels decreased following LSG, and liver histopathology results improved[196]. Similar observations were made in a different study, which concluded that weight reduction after LSG was associated with altered levels of anti-inflammatory adipokines and better glucose metabolism[197]. Šebunova *et al*[169] observed that resistin was markedly higher after LSG than after RYGB, however, the decrease from the preoperative period was not significant. Farey *et al*[198] found that postoperative resistin levels exhibited a reducing trend which was not statistically significant, and that resistin levels of obese patients were lower than those of non-obese controls.

Additionally, a meta-analysis revealed that weight reduction surgery had no pronounced impact on resistin levels[199]. Presently available studies seem not to support the hypothesis that LSG regulates resistin levels to facilitate T2DM remission. However, the various limitations of such studies should be considered, particularly with regard to small sample sizes and the fact that resistin is not consistently highly expressed in obese people. Further research is required to determine whether preoperative resistin levels are generally within a normal range to more accurately assess its impact on T2DM.

Chemerin

Chemerin was found to be highly expressed in human WAT in 2007. Chemerin is a novel adipokine that binds to the orphan G protein-coupled receptors chemokine-like receptor 1, chemokine receptor-like 2, and G protein-coupled receptor 1 to exert its potential autocrine and paracrine effects[200,201]. It may have a role in energy balance and metabolism *in vivo* and is linked to adult obesity, T2DM, and metabolic syndrome, according to recent research[202]. Most respective studies found that people with poor glucose homeostasis had higher serum chemerin levels and that this increase was inversely linked with glycemic control parameters[203]. A meta-analysis suggested a marked decline in chemerin levels after BS, however, various surgical methods were not distinguished[199]. Terra *et al*[153] reported a significant decrease in chemerin 12 mo after LSG, compared with the baseline levels, in a pattern similar to that after RYGB. Similar findings were reported by Jouan *et al*[204], who discovered a decrease in chemerin after surgery and suggested that chemerin may be utilized as a predictor of a postoperative inflammation; however, the changes in chemerin after LSG were not uniform. The findings of Cătoi *et al* [205] did not reveal any significant differences in chemerin six months after LSG. Chemerin is a relatively novel adipokine; thus, little information is available, and most conclusions originate from meta-analyses. Fundamental research is thus required to understand the mechanisms of action of chemerin acts, particularly with regard to T2DM. The limited available data do not support a link between chemerin and improved glucose metabolism after LSG.

Omentin-1

Omentin-1 is the primary circulating form of omentin, also referred to as intelectin-1, which is mainly expressed in visceral adipose tissue and exerts endocrine effects resembling those of hormones[206]. Omentin-1 increases insulin sensitivity, which is key in maintaining the body's metabolism. In addition,

it also has anti-inflammatory properties through the intracellular Akt/AMPK/NF-B and mitogen-activated protein kinase signaling pathways[207]. Glucose/insulin and FGF21 affect how omentin-1 is regulated, with glucose/insulin decreasing its expression and secretion and FGF21 increasing it[208, 209]. Omentin-1 expression profiles of obese and T2DM patients showed that its expression and secretion were suppressed in patients suffering from obesity[210], T1DM[211], T2DM[212], and metabolic syndrome. In addition, the chromosomal area of omentin-1 is linked to T2DM in certain groups. Thus, this gene may be associated with T2DM susceptibility[213]. Increased circulating omentin-1 Levels and decreased fecal omentin mRNA after LSG may contribute to surgery-induced metabolic improvement and weight reduction[214]. Sdralis *et al*[215] proposed that LSG combined with omentotomy reduced the expression of omentin-1, but LSG alone increased it, and a low-calorie diet had no significant effect on omentin-1. The pattern of omentin-1 expression after LSG is intriguing, however, as omentin-1 is influenced by glucose/insulin and FGF21, it is unclear whether the reduction in blood glucose under T2DM remission would prevent the inhibition of omentin-1, causing it to increase, or whether the higher omentin-1 Levels affected T2DM remission. Omentin-1-based medication may be an emerging option for treating obesity and T2DM, considering the link between omentin-1 and IR. However, the mechanisms of action of omentin-1 during surgical operations are unclear.

Visfatin

Visceral fat secretes the adipokine visfatin, which has effects similar to those of insulin[216]. Visfatin interacts with insulin receptors during gluconeogenesis to increase glucose absorption in liver and muscle tissue, thus lowering blood sugar levels[217]. Further, it supports the effects of insulin by causing the phosphorylation of insulin receptors 1 and 2[218]. Additionally, the autocrine activity of visfatin in the liver enhances insulin sensitivity[219], and it also works on the hypothalamus in the center to influence insulin release and reduce IR[220]. According to studies, visfatin contributes to IR and T2DM in a dose-dependent manner, and obese patients with T2DM showed higher intraserum levels of visfatin than obese patients without T2DM[221]. However, only few studies could be identified that examined how LSG affected visfatin, one of which found no evidence of a substantial change in visfatin after LSG[222]. Similar conclusions were drawn in a meta-analysis, which showed that BS had no marked impact on visfatin expression or secretion[195]. Animal experiments produced similar results [223]. Visfatin has a beneficial effect on T2DM or decreased glucose tolerance because of its insulin-like activity. However, uncertainty remains regarding how LSG affects visfatin levels and how visfatin contributes to T2DM remission following LSG.

Retinol binding protein 4

Retinol binding protein 4 (RBP4) is an adipokine secreted by WAT. The primary function is to transport retinol, the active metabolite of vitamin A, from the liver to target tissues. High levels of RBP4 are associated with developing metabolic diseases such as obesity, IR, metabolic syndrome, and T2DM [224]. In obesity, abnormal levels of RBP4 produce both local and systemic effects (retinol homeostasis and transport *in vivo*)[225]. It exacerbates the inflammatory state in obesity *in vivo* by activating Toll-like receptor (TLR) 2 and TLR4/myeloid differentiation protein 2 receptor complexes in macrophages[226]. In T2DM, RBP4 is associated with IR and the progression of several T2DM co-morbidities, such as diabetic nephropathy and diabetic retinopathy[227]. Whether RBP4 is elevated in obesity is controversial, as Yang *et al*[228] found higher serum RBP4 Levels in obese individuals than in lean individuals. However, similar alterations were not found in the study by Korek *et al*[229] What is certain is that there is a correlation between elevated blood RBP4 Levels and the incidence of IR, serum lipid levels, and anthropometric parameters[224]. Wang *et al*[230] reported a significant decrease in RBP4 after LSG and concluded that RBP4 Levels positively correlated with BMI, glucose, fasting C-peptide, and HOMA-IR. In another study, the authors found that RBP4 decreased after LSG in children and adolescents[231]. However, some studies have also shown that LSG did not significantly affect RBP4 Levels[232,233]. In addition, Jüllig *et al*[234] found that RBP4 decreased more in patients after RYGB than after LSG. Fewer studies have been conducted on the effect of LSG on RBP4, and only sporadic studies have been reported; therefore, it is impossible to determine the changes involved. However, it is worth affirming that RBP4, as a specific adipokine, plays an important role in T2DM, and targeting RBP4 may become a potential therapeutic strategy.

GM, BAS, AND THEIR INTERACTIONS

GM

The human gut contains a unique variety of microbes, commonly known as the GM, which comprises approximately 3 million non-redundant microbial genes[235]. The GM may impact host metabolic functions, such as energy generation, steroid hormone synthesis, and bile salt metabolism, and they are intricately related to the development of metabolic diseases[236]. By increasing energy absorption from food, alterations in the GM, in particular, plays a significant role in the onset and progression of obesity

and T2DM[237]. In obese people, the GM exhibits particular traits, including altered microbial gene abundance and ecological dysregulation which is linked to inflammation, increased body weight and fat mass, and T2DM[238]. Therefore, modifying the GM may be an option for treating T2DM and obesity. Studies have demonstrated that oral administration of improved GM to rats with metabolic syndrome increased insulin sensitivity[239]. Whether SG causes specific changes in the GM that contribute to improving metabolic disorders remains unclear. Tabasi *et al*[240] observed changes in the diversity and composition of the GM three months after LSG, and long-term follow-up studies showed that most changes remained for one year after surgery, indicating that SG elicits rapid and sustainable changes [241]. The alterations in GM due to RYGB and SG were varied, with RYGB increasing the relative abundances of the phyla Firmicutes and Actinobacteria but reducing those of Bacteroidetes, whereas SG increased Bacteroidetes abundances. Of note, Roseburia species abundance was increased in all patients who achieved T2DM remission, which was common to SG and RYGB[241]. Changes in GM after LSG occur universally, which has been validated in several studies[242,243]. This contributes to the various concerns regarding the degree to which the GM may impact the outcome of LSG and whether specific changes in the particular flora play a dominant role in improving T2DM or obesity. Surgery based on changed GM or fecal transplantation therapy may open new avenues for treating T2DM and obesity.

BAs

BAs are planar amphiphilic molecules with a carboxyl tail that are generated in the liver[244]. Diet regulates the synthesis, secretion, and circulation of BAs. In addition to the typical role of lipid absorption, BAs operate as signaling chemicals through two key receptors, *i.e.*, Farnesoid X receptor (FXR) and Tekeda-G-protein receptor 5 (TGR5)[245]. The hepatic-intestinal cycle occurs when BAs are released into the duodenum after eating, and most of them are reabsorbed and transported back to the liver after they reach the ileum[246]. Current studies showed that BAs play a significant function in controlling lipid, glucose, and energy metabolism and that obesity and T2DM are associated with dysregulated BAs homeostasis *in vivo*[247]. Most respective studies confirmed that BAs alterations are similar in obese, T2DM, and IR patients, who show higher fasting BA levels than healthy controls[248]. However, this variation is not uniform, and many studies concluded that BA levels are not significantly altered[249]. The effect of LSG on BA levels is also somewhat controversial. Yang *et al*[133] revealed that BA levels exhibited a transitory decrease following LSG and thereafter a progressive increase. In contrast, following RYGB, BA levels show a consistently increasing trend. While Eiken *et al*[250] discovered higher BA concentrations after RYGB, increased inflow of BAs into the small intestine and more rapid release, this did not occur after LSG. Cătoi *et al*[251] examined the relationship between IR and BAs after LSG and found no significant changes in BA levels and HOMA-IR in the very early period (1 wk) after surgery. However, one month postoperatively, total BA levels increased, HOMA-IR decreased, and there was a negative correlation between them. In a different study, there was a link between higher BAs levels and better-glycated hemoglobin. Fasting and postprandial levels of total, secondary, and unconjugated BAs were higher after LSG[130]. Wang *et al*[252] discovered that after SG, total BA levels increased, and the fraction of 12-hydroxylated BAs was reduced in a diabetic rat model. This alteration may be fundamental to improved insulin sensitivity after SG. There are some differences between RYGB and LSG with regard to changes in total BAs after BS. One possible explanation for these differences is that RYBG entails changes in the structure of the gastrointestinal tract that affect the hepatic-intestinal circulation of BAs, whereas LSG does not. LSG and total blood BA levels and BA composition are unarguably linked; however, further research is required to help understand how certain BA species affect postoperative variations in LSG.

Interactions of BAs and GM

BAs and the GM interact in both directions (Figure 2). In the distal small intestine and colon, where most of the GM occurs, hydroxylation and dihydroxylation occur, through which the GM regulates the composition of BAs and controls the generation of secondary BAs[253]. By modifying the composition structure of BAs, the GM may further regulate FXR and TGR5 functions[254]. Biological agents that affect the GM can alter the BA profile[255], and BAs can affect the GM due to their antimicrobial effects and impact on intestinal mucosal integrity[256]. In conclusion, elucidating the relationship between BAs and the GM may provide a better understanding of the variability in weight reduction and enhanced glucose metabolism between RYGB and LSG. The stronger influence of RYGB on the GM owing to changed physiological channels induces alterations in BAs, whereas this effect is apparently minor after LSG.

CONCLUSION

LSG is an effective therapy option for the worrying pandemic of obesity and T2DM. LSG entails several therapeutic mechanisms that enhance glucose homeostasis and IR without relying on weight reduction. The gut-brain, gut-adipose tissue, gut-hepatic, gut-pancreatic, and gut-muscle axes are some of these putative entities. These insights may provide novel avenues for T2DM treatment targets focused on the

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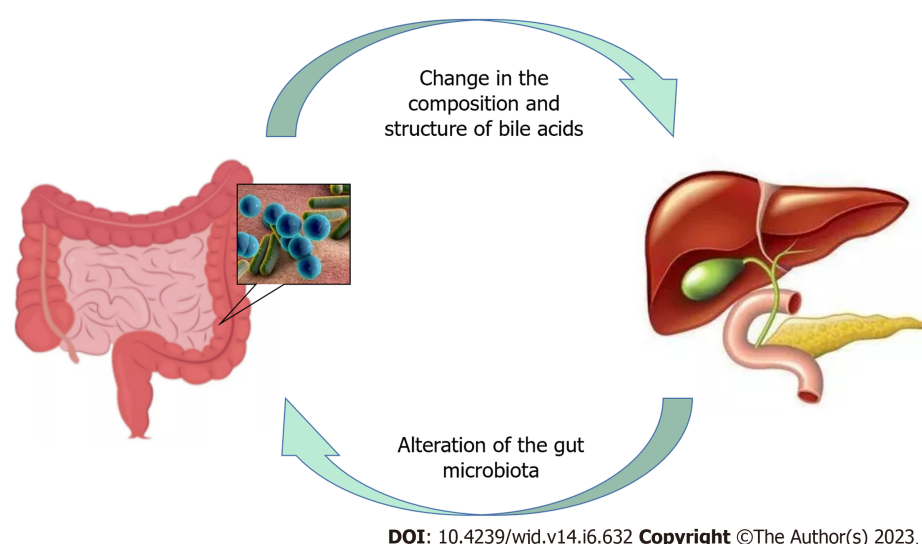
Figure 2 Interaction between gut microbiota and bile acids.

gut. Overall, the understanding of how LSG works to treat T2DM has considerably advanced, however, further research is required. Additionally, while obese and T2DM patients may benefit from LSG, some hazards must be carefully considered, such as higher levels of certain GHs that may cause postprandial hyperinsulinemic hypoglycemia and decreased appetite, leading to malnutrition in non-overweight individuals.

ACKNOWLEDGEMENTS

Fa-Shun Liu would like to express his gratitude to his wife Yan-Wen Ran for the support she provided.

FOOTNOTES

ORCID number: Fa-Shun Liu 0000-0002-6825-9559; Song Wang 0000-0003-3053-9229; Xian-Shan Guo 0000-0003-1288-4061; Hong-Ya Zhang 0000-0002-8046-0829; Zhen Li 0000-0002-6147-7545.

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Genetics of diabetes

Shiwali Goyal, Jyoti Rani, Mohd Akbar Bhat, Vanita Vanita

Shiwali Goyal, Department of Ophthalmic Genetics and Visual Function Branch, National Eye Institute, Rockville, MD 20852, United States

Jyoti Rani, Vanita Vanita, Department of Human Genetics, Guru Nanak Dev University, Amritsar 143005, Punjab, India

Mohd Akbar Bhat, Department of Ophthalmology, Georgetown University Medical Center, Washington DC, DC 20057, United States

Corresponding author: Vanita Vanita, PhD, Professor, Department of Human Genetics, Guru Nanak Dev University, GT Road, Amritsar 143005, Punjab, India. vanita.humangenetics@gmail.com

Abstract

Diabetes mellitus is a complicated disease characterized by a complex interplay of genetic, epigenetic, and environmental variables. It is one of the world's fastest-growing diseases, with 783 million adults expected to be affected by 2045. Devastating macrovascular consequences (cerebrovascular disease, cardiovascular disease, and peripheral vascular disease) and microvascular complications (like retinopathy, nephropathy, and neuropathy) increase mortality, blindness, kidney failure, and overall quality of life in individuals with diabetes. Clinical risk factors and glycemic management alone cannot predict the development of vascular problems; multiple genetic investigations have revealed a clear hereditary component to both diabetes and its related complications. In the twenty-first century, technological advancements (genome-wide association studies, next-generation sequencing, and exome-sequencing) have led to the identification of genetic variants associated with diabetes, however, these variants can only explain a small proportion of the total heritability of the condition. In this review, we address some of the likely explanations for this "missing heritability", for diabetes such as the significance of uncommon variants, gene-environment interactions, and epigenetics. Current discoveries clinical value, management of diabetes, and future research directions are also discussed.

Key Words: Type 1 diabetes; Type 2 diabetes; Gestational diabetes mellitus; Maturity-onset diabetes of young; Genome-wide association studies; Common variants; Rare variants

Core Tip: Diabetes pathogenesis encompasses genetic, epigenetic, and environmental variables and their interactions. To date, the examined common variations can explain just a small portion of the heritability of diabetes. Furthermore, the technique of integrating the associated variants as a type of genetic risk score does not accurately predict diabetes risk. As a result, the trend for genetic risk factors for diabetes is shifting from common to rare variants. Aside from genetic variables, systemic data from other transomics such as epigenomics, transcriptomics, proteomics, metabolomics, and metagenomics will contribute to a better understanding of genetic determinants in the progression of metabolic illnesses like diabetes. Technological, computational, and collaborative developments continue to uncover novel genetic diabetes risk factors. There are high prospects for tailored diabetes treatment in the future, based on increased knowledge of the molecular genetic profile of the patients.

INTRODUCTION

Diabetes mellitus (DM) is a set of diverse metabolic illnesses characterized by disturbances in the metabolism of glucose, resulting in hyperglycemia and glucose intolerance. Diabetes can occur either by the failure of the body to produce insulin, resistance to the action of insulin, or both[1,2]. DM is one of the most common endocrinological disorders worldwide. Its prevalence is rising because of physiological risk factors such as socioeconomic level, stress, obesity, hyperlipidemia, and hypertension. In addition to these, changes in behavioral patterns such as unhealthy lifestyles and eating habits can contribute significantly to the pathogenesis of diabetes[3]. DM has a devastating effect on different organs of the body such as the heart, kidneys, nerves, and eyes, and can lead to the development of various long-term microvascular or macrovascular complications[4,5]. The rapid global increase in instances of diabetes, which affects people's life expectancy and quality of life, places a significant public health burden on society[6].

CLASSIFICATION OF DIABETES MELLITUS

DM can be broadly classified into four types (Figure 1) *i.e.*, type 1 DM (T1DM), type 2 DM (T2DM), gestational DM (GDM), and maturity-onset diabetes of young (MODY)[7]. Of these, T2DM is the most prevalent form of diabetes accounting for 90% of all cases worldwide.

Type 1 diabetes mellitus

T1DM is also known as insulin-dependent DM (IDDM) or juvenile-onset diabetes. T1DM is caused by the autoimmune destruction of pancreatic beta cells by a T-cell-mediated inflammatory response, resulting in reduced insulin production. T1DM accounts for around 5%-10% of the individuals diagnosed with diabetes and approximately 80%-90% of cases with diabetes among children and adolescents[8]. The interaction between T-lymphocytes and autoantigens causes beta-cell death. In newborns and children, the rate of beta cell loss is relatively variable with rapid progression. Adults are more likely to develop the slowly progressive form, commonly known as latent autoimmune diabetes in adults (LADA). At this stage, the body secretes little or no insulin, and patients frequently become dependent on insulin for survival[2,9].

Type 2 diabetes mellitus

T2DM is the most common type of diabetes, accounting for almost 90% of all cases globally. T2DM is characterized by insulin insensitivity caused by insulin resistance, poor insulin production, and pancreatic beta-cell destruction. The increased demand for insulin in the target tissues caused by insulin resistance could not be met due to beta cell abnormalities, resulting in hyperglycemia[10]. T2DM is a complex condition characterized by a combination of genetic as well as environmental variables, such as stress, obesity, and lack of physical activity[11].

Gestational diabetes mellitus

Gestational diabetes is most common in pregnant women and accounts for about 7% of all pregnancy cases. Females having a history of GDM are 10 times more likely to develop postpartum T2DM, cardiovascular disease, and metabolic perturbation in the future[12]. Furthermore, children of pregnant women with gestational diabetes are at risk of anomalies related to glucose metabolism and have a 40 to
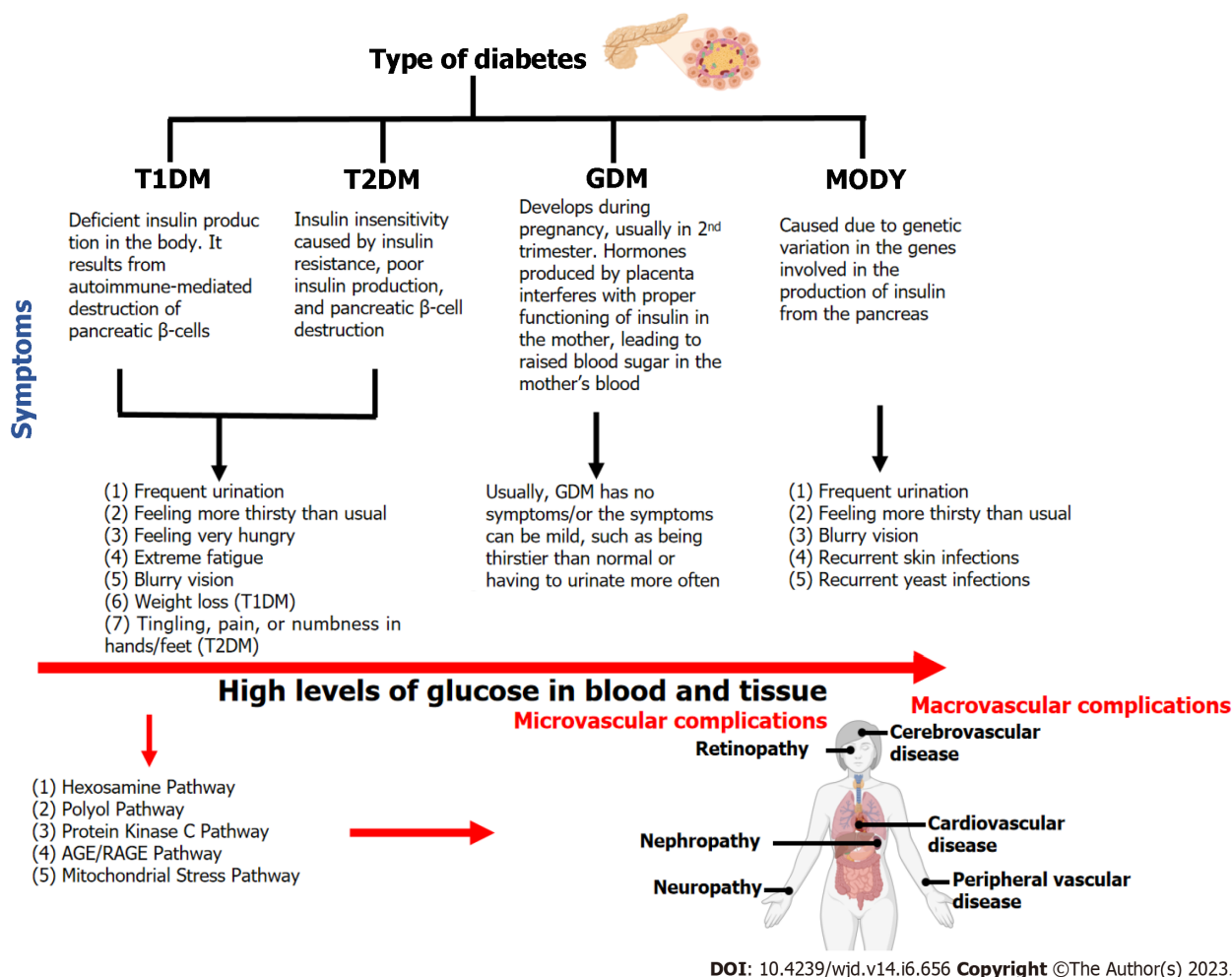
Figure 1 Types of diabetes and their symptoms. Hyperglycemia and potential metabolic pathways in the pathogenesis of diabetic complications (microvascular and macrovascular) are also indicated. AGE: Advanced glycation end-products; RAGE: Receptor for advanced glycation end-products; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; GDM: Gestational diabetes mellitus; MODY: Maturity-onset diabetes of young.

60 percent chance of getting diabetes in adulthood[13]. Women with a family history of diabetes and obese women are more likely to develop gestational diabetes[14].

Maturity onset diabetes of young
MODY, a monogenic variant of type 2 diabetes, has an autosomal dominant inheritance pattern and is characterized mostly by insulin secretion abnormalities, however, with normal insulin action[15]. MODY generally occurs before the age of 25 years or during childhood[2]. Roughly 2%-5% of type 2 diabetes patients have been estimated to have MODY. Different types of MODY are classified based on underlying genetic defect: MODY1 (*HNF4A*); MODY2 (*GCK*); MODY3 (*HNF1A*); MODY4 (*PDX1*); MODY5 (*HNF1B*); MODY6 (*NEUROD1*); MODY12 (*ABCC8*), and MODY13 (*KCNJ11*).

ATYPICAL DIABETES MELLITUS

There are two atypical types of DM: LADA and ketosis-prone DM (KPDM), both of which are prone to misdiagnosis, leading to ineffective management.

Latent autoimmune diabetes of adults
LADA is a kind of autoimmune diabetes that resembles T1DM, but the onset is during adulthood, and it progresses slowly toward absolute insulin insufficiency than classical childhood-onset T1DM, which requires prompt exogenous insulin therapy[16]. Approximately 2%-12% of all DM patients may have LADA[17]. Most LADA patients do not require insulin at the time of diagnosis; nevertheless, they do have diabetes-specific autoantibodies. As a result, they have characteristics of both T1DM and T2DM and are at risk of being misdiagnosed as having T2DM[18]. According to studies from China, Korea, India, and the United Arab Emirates, the prevalence of LADA is 5.7%, 4.4% to 5.3%, 2.6% to 3.2%, and

2.6%, respectively[19]. Usage of clinical risk tools (age of onset of diabetes < 50 years, acute symptoms of hyperglycemia at the time of onset, body mass index < 25 kg/m^2, family history or personal history of autoimmune disease), and evaluation of C-peptide level can help identify individuals at higher risk of LADA in adults[19].

Ketosis-prone diabetes mellitus

Diabetic ketoacidosis is a potentially fatal but treatable complication of DM that is characterized by hyperglycemia, metabolic acidosis, and ketonemia as a result of absolute or relative insulin insufficiency [20]. Although the actual prevalence of KPDM is unknown, men have a higher prevalence than women [21]. Patients with KPDM typically show acute and very recent history (mostly < 4 wk) of hyperglycemic symptoms such as polyuria, polydipsia, and weight-loss[22,23].

GLOBAL PREVALENCE OF DIABETES MELLITUS

Diabetes is one of the fastest-growing global health emergencies of the 21st century (Figure 2). Diabetes affected around 537 million people in 2021, and this number is projected to reach 643 million by 2030 and 783 million by 2045, which is a nearly 46% increase in its prevalence[24]. Middle-income countries are expected to see the greatest percentage increase in the prevalence of diabetes, followed by high- and low-income countries. In 2021, there were approximately 8.4 million individuals worldwide with T1DM, of which 1.5 million were younger than 20 years of age. In 2040 the prevalence of T1DM has been predicted to increase to 13.5-17.4 million (60%-107% higher than in 2021)[25]. The frequency of the most common type of DM *i.e.*, T2DM varies substantially by region, with low and middle-income countries accounting for almost 80% of all T2DM cases[26]. This variance in diabetes incidence across the globe may be attributable to environmental as well as lifestyle factors apart from underlying genetic components. Globally, the prevalence of GDM varies greatly (from 1% to 28%) depending on demographic variables (*e.g.*, maternal age, socioeconomic status, race or ethnicity, or body composition), screening methods, and diagnostic criteria. The estimated prevalence of MODY is 1 in 10000 for adults and 1 in 23000 for children.
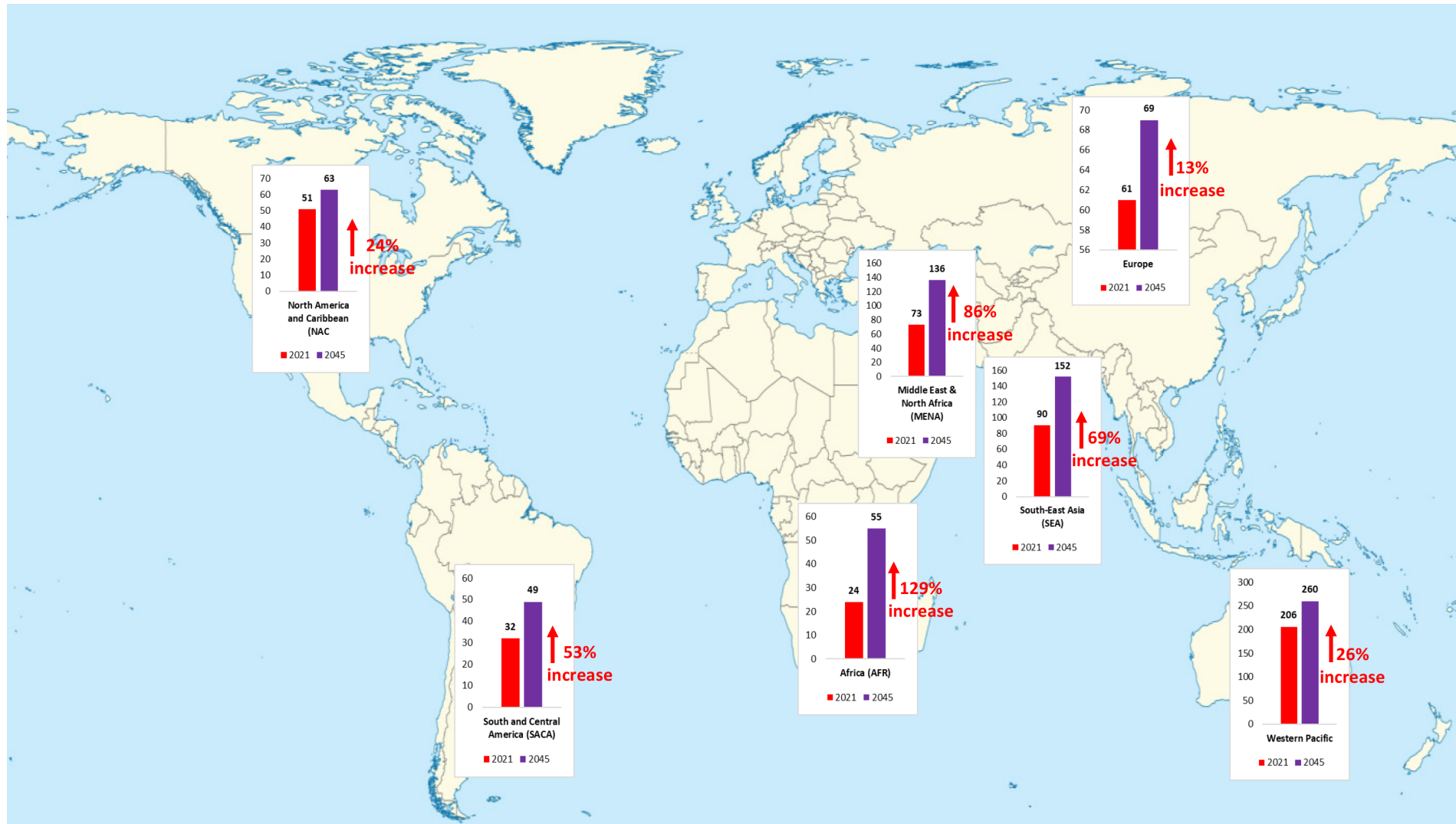
PATHOGENESIS OF DIABETES MELLITUS

The pathogenesis of type 2 DM is influenced by eight key abnormalities described collectively as "the ominous octet"[27] (Figure 3). Reduced insulin secretion, decreased incretin action, increased lipolysis, increased glucose reabsorption, decreased glucose uptake, neurotransmitter dysfunction, increased hepatic glucose synthesis, and increased glucagon secretion are examples of these[27,28]. Therapy options for T2DM should target these documented pathophysiological abnormalities while also using a patient-centered approach that incorporates aspects other than glycemic control, such as lowering overall cardiovascular risk[29,30]. Recent research has indicated that during the progression of T2DM, pancreatic β-cells undergo dynamic compensation and decompensation processes, with metabolic stressors such as endoplasmic reticulum stress, oxidative stress, and apoptosis acting as major regulators of the β-cell dynamics[31].

T1DM is characterized by the autoimmune death of pancreatic beta cells produced by a T-cell-mediated inflammatory response, which results in decreased insulin production (Figure 3). On the other hand, in GDM, glucose intolerance develops usually in the second trimester which results in adverse impacts on both mother and offspring (Figure 3). MODY is caused by mutations in the *GCK*, *HNF*, and *NEUROD1* genes, which are involved in glucose metabolism, insulin control, glucose transport, and fetal pancreas development.

Several pathways play a significant role in causing the microvascular and macrovascular complications associated with T2DM. Hexosamine biosynthetic pathway is implicated in the development of insulin resistance and diabetic vascular problems. It has been reported that hyperglycemia increases the production of transforming growth factor-beta, a prosclerotic cytokine implicated in the development of diabetic nephropathy[32]. The polyol pathway is a two-step metabolic mechanism that converts glucose to sorbitol and then to fructose[33,34]. It has long been assumed that the polyol pathway is almost silent under normal physiological conditions but becomes active and detrimental under hyperglycemic conditions. The protein kinase C pathway in diabetes promotes vascular contractility in an endothelium-independent way through K$^+$ channel inactivation and Ca^{2+} sensitization of myofilaments in vascular smooth muscle cells[35]. The binding of advanced glycation end products to its receptor activates a range of signaling pathways, which further enhances oxidative stress, hence leading to nerve cell damage and apoptosis[36].

Figure 2 Predicted percentage increase in the global prevalence of diabetes mellitus from 2021 to 2045[24].
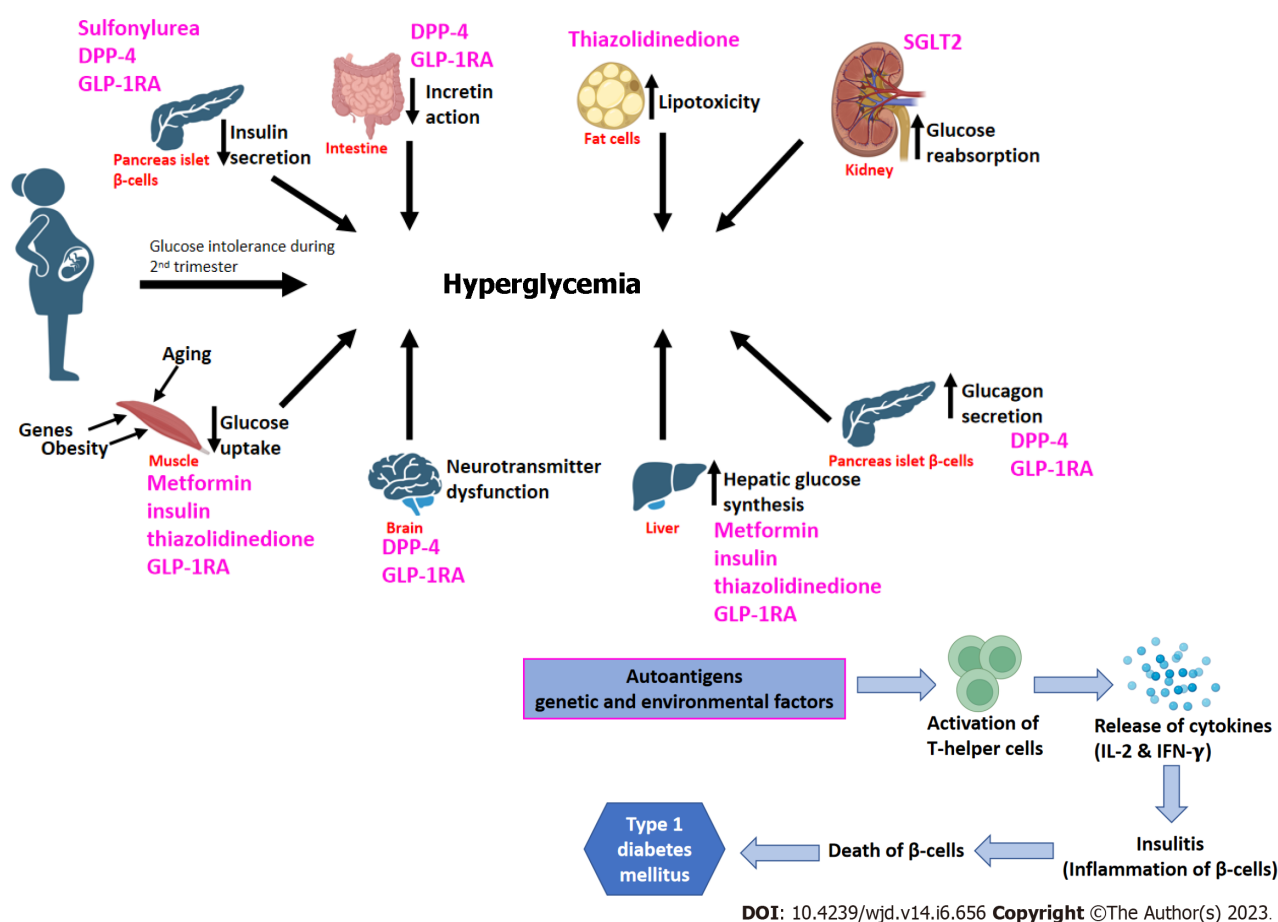
Figure 3 Pathogenesis of gestational diabetes mellitus, type 2 diabetes mellitus-ominous octet, and type 1 diabetes mellitus.
Pharmacological glycemic management targets have also been shown here. DPP-4: Dipeptidyl peptide-4 inhibitor; GLP-1RA: Glucagon-like peptide-1 receptor agonist; SGLT2: Sodium-Glucose co-transporter 2 inhibitor; IL-2: Interleukin-2; IFN-γ: Interferon gamma.

IDENTIFICATION OF DIABETES SUSCEPTIBILITY GENES

Family and twin studies have reported 20%-80% of heritability in diabetes. First-degree relatives of people with T2DM are three times more likely to get the disease than people without a positive family history[37]. Even though diabetes from both the maternal and paternal side increases the risk of acquiring diabetes, the Framingham Offspring research reported that offspring with maternal diabetes had a slightly higher risk of impaired glucose tolerance than those with paternal diabetes[24]. Multiple twin concordance studies in T2DM found that monozygotic twins had a greater concordance rate than dizygotic twins, indicating that the condition has a significant genetic component[37]. On the other hand for T1DM, monozygotic twins have a concordance rate of 40%-50% in population-based twin studies [38]. The following methods have been used to identify the diabetes risk gene.

Genetic linkage studies

Linkage analysis is based on the principle that genetic sequences located on the same chromosome tend to be inherited together and are not separated during meiotic homologous recombination. It is typically used in family studies to determine the position of an associated variant(s)[39,40]. Linkage studies have successfully uncovered genetic variations that cause monogenic diseases such as MODY[41]. In 1996, using linkage analysis, major histocompatibility complex loci (HLA) on chromosome 6 were identified as the genetic susceptibility loci for T1DM[42]. In 2004, the calpain-10 gene (*CAPN10*) on chromosome 2 was identified as the cause of T2DM using genome-wide screening and positional cloning[43,44]. *TCF7L2*, the now well-known T2DM gene, was mapped to chromosome 10 in a Mexican-American group in the year 1999 and has been replicated several times in T2DM genome-wide association studies (GWAS)[45,46]. *TCF7L2* plays an important role in the Wnt/β-catenin signaling pathway and helps in regulating the expression of genes in lipid metabolism in adipocytes and glucose-induced insulin exocytosis.

Candidate gene association studies

It is a hypothesis-driven method in which candidate genes are chosen based on prior knowledge such as

a gene's biological function, position, or probable significance about a given phenotype[47]. This method is usually more suitable in studies where individuals are unrelated[48]. Candidate gene studies revealed an association between T2DM and insulin receptor substrate 1 (*IRS1*), peroxisome proliferator-activated receptor gamma (*PPARG*), and insulin receptor substrate 2 (*IRS2*), Wolfram syndrome 1 (wolframin) (*WFS1*), potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*), HNF1 homeobox A (*HNF1A*), and HNF1 homeobox B (*HNF1B*)[49]. By association studies for T1DM, four non-HLA genes with established risk loci [*HLA*, *INS* (insulin), *CTLA4* (cytotoxic T-lymphocyte antigen 4), *PTPN22*][50] could be identified. Of all the genes identified for gestational DM; *TCF7L2*, *MTNR1B*, *CDKAL1*, *IRS1*, and *KCNQ1* candidate genes are the most common, whereas other identified genes are ethnic-specific. On the other hand, MODY is inherited in an autosomal dominant pattern and manifests itself as a result of mutations in transcription factor genes such as *HNF4* (hepatocyte nuclear factor), *HNF1*, *IPF1* (insulin promoter factor), and neuro-D1[51,52].

Genome-wide association studies

GWAS are large-scale hypothesis-free investigations that entail the fast scanning of genetic variants (SNPs on genotyping arrays) across the complete human genome to uncover unique genetic associations with a certain trait[53]. The initial T2DM-related GWAS studies identified hematopoietic expressed homeobox (*HHEX*), solute carrier family 30 member 8 (*SLC30A8*), cyclin-dependent kinase inhibitor 31 2A/2B (*CDKN2A/2B*), insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), CDK5 regulatory subunit associated protein 1 Like 1 (*CDKAL1*), and FTO alpha-ketoglutarate (*FTO*)[54-58]. Approximately 250 significant susceptibility loci for T2DM have been identified to date (https://www.ebi.ac.uk/gwas/efotraits/MONDO_0005148). On the other hand, for T1DM by GWAS more than 60 loci have so far been discovered (https://www.ebi.ac.uk/gwas/efotraits/MONDO_0005147), revealing the pathways underlying the disease, and overlaps with autoimmune diseases[59]. GWAS in T1DM has not only verified the previously reported T1DM loci but also uncovered several novel variations, such as those near the *KIAA0350* (*CLEC16A* approved symbol)[60] gene and with *UBASH3A* (ubiquitin-associated and SH3 containing A)[61]. To our knowledge, to date, only three GWAS have been conducted for GDM[62-64]. Kwak *et al*[62] identified two significant GDM variants, rs7754840 and rs10830962 in the intronic region of *CDKAL1*, and upstream of *MTNR1B*, respectively. On the other hand, Wu *et al*[63] identified 23 SNPs in four genes: *CTIF*, *CDH18*, *PTGIS*, and *SYNPR* to be associated with GDM. Recently, Pervjakova *et al*[64] through multi-ancestry meta-analysis reported five loci (mapping to/near *MTNR1B*, *TCF7L2*, *CDKAL1*, *CDKN2A-CDKN2B*, and *HKDC1*) through genome-wide association studies for GDM. Using a meta-analysis approach, the genetic architecture of T1DM and T2DM has been determined in many populations with different ethnic backgrounds[65-74].

There are many challenges to the GWAS approach. The current GWAS genotyping arrays are based on HapMap and the 1000 genome project dataset, and these are designed to target common SNPs (MAF > 5%). As a result, the prior GWAS did not directly investigate rare variants for an association with the trait[75]. Also, the observed variants that are linked to the trait may not be the causal variations, but rather be in linkage disequilibrium with the causal variants. Furthermore, since the variant is often located outside the coding regions and may affect genes and regulatory elements at a distance, it is usually difficult to understand how the variant affects the trait.

Genome-wide rare variants association studies

The 'common disease, rare variant' hypothesis, in contrast to the standard 'common disease, common variant' paradigm, says that many rare genetic variations with relatively high penetrance play a significant influence in the elevated risk of common diseases[76]. Huyghe *et al*[77] for the first time in 2013 investigated the significance of low-frequency variants (minor allele frequency < 5%) associated with the risk of T2DM or T2DM-related traits using the Illumina exome array technique. Two low-frequency variants in *SGSM2* and *MADD* were reported to be associated with fasting proinsulin concentrations and three novel variants in *TBC1D30*, *KANK1*, and *PAM* genes were reported with proinsulin or insulinogenic index. Later in 2014, Steinthorsdottir *et al*[68] using an exome sequencing technique in the Icelandic population, reported three more T2DM-associated low-frequency variants in *CCND2*, *PAM*, and *PDX1*. In the following years, rare variants in *MTNR1B*, *HNF1*, and *G6PC2* genes were also reported to be associated with T2DM or T2D-related traits[78]. Nejentsev *et al*[79] reported four rare variants (rs35667974, rs35337543, rs35732034, and rs35744605) in *IFIH1*, a gene previously discovered in T1DM GWAS. Additionally, a cluster of rare detrimental variations in *PTPN22* was identified for T1DM, comprising two novel frameshift mutations (rs538819444 and rs371865329) and two missense variants (rs74163663 and rs56048322)[80].

EPIGENETIC ALTERATIONS IN T2DM

The term "epigenetics" refers to heritable alterations in gene function that occur without a change in the nucleotide sequence. Epigenetic changes can be inherited from one cell generation to the next and in some cases, can be inherited through the generations. Epigenetic changes can also develop during life,

either randomly or in response to environmental stimuli, impacting the effects of genetic variants and so acting as a gene-environment interaction mechanism. Both DNA methylation and histone modifications can amend the response of our genome to the environment during life. The involvement of intrauterine DNA methylation and imprinting in the programming of diabetogenic effects later in life has received significant interest in the etiology of the T2DM[81]. An intriguing study by Dabelea *et al*[82] found that intrauterine diabetes exposure increased the incidence of diabetes and obesity in offspring compared to siblings born before their mothers' diabetes onset. However, the precise mechanism underlying this maternal impact is unknown. Some studies have suggested a role of epigenetic regulation of genes involved in energy metabolism, appetite control, and -cell function, such as *PPARA*[83], *LEP*[84], and pancreatic and duodenal homeobox 1 (*PDX1*)[85].

MICRORNAS

MicroRNAs (miRNAs) have emerged as promising novel biomarkers for T2DM and related problems due to their metabolic stability and abundance in various body fluids including blood and cerebrospinal fluid. miRNAs are a class of endogenous, small (18-25 nucleotide) RNA that regulates many cellular activities by suppressing gene expression[86]. According to recent research, differential concentrations of circulating miRNAs (Table 1)[87-128] may offer the intriguing potential for diabetes (T1DM, T2DM, MODY, and GDM) diagnosis, prognosis, and treatment monitoring.

POLYGENIC RISK SCORES FOR T2DM

Since, T2DM is the most common form of diabetes, hence most of the polygenic risk scores (PRSs) studies have been performed on T2DM. GWAS investigations have enabled the development of PRSs or genetic risk score (GRS) that assess an individual's lifetime genetic risk for various diseases. Several studies on coronary artery disease have been reported[129-132], however, there is a scarcity of reports on the prediction models for diabetes (T1DM, T2DM, and GDM). The area under the receiver operating characteristics curve is a measure of the prediction accuracy of the constructed PRS[133]. One of the first research estimated a T2DM GRS using a combination of 18 loci and reported that genetic information only marginally improved risk prediction when paired with standard clinical risk factors such as age, gender, or diabetes family history[134-136] (Table 2). There has been a rise of interest in GRS in recent years, utilizing many more loci reported from large-scale, multi-ancestry cohorts. T2DM GRS studies from large datasets[137-139] reported that GRS constructed from multi-ethnic computed weights indicated a marginal increase in predictive power as compared to single-ancestry computed weights, the reason might be heterogeneity across different ancestries (Table 2)[140-149].

PRSs have also been demonstrated to predict pre-diabetes and T2DM in women with a history of GDM (Table 3)[150-153]. Some studies have found that using a PRS in conjunction with traditional T2DM risk factors improves discrimination of the risk of pre-diabetes in women with prior GDM, potentially giving more accurate tools for the prediction of future T2DM.

GRS, on the other hand, may have a role in recognizing high-risk patients before clinical risk markers become apparent. It needs to be shown whether GRS data can drive preventive therapy to meaningfully reduce rates of future incident T2DM.

LIFESTYLE MODIFICATIONS, ENVIRONMENTAL FACTORS, AND MANAGEMENT OF DIABETES MELLITUS

In the long term, the pharmacological strategy for treating diabetes may be only partially effective. Major changes in patients' lifestyles (change in physical activity, dietary alteration, stress management, and improved sleeping patterns), along with treatments through pharmacological techniques, are required to ensure optimal disease management. Self-monitoring of blood glucose is an excellent tool for monitoring glycemic status. Current American Diabetes Association (ADA) guidelines urge its use in all patients with T1DM, T2DM, or any other form of diabetes (*e.g.*, gestational diabetes) that requires numerous subcutaneous insulin injections[154]. Continuous glucose monitoring systems *i.e.*, Dexcom G6, Frestyle Libre 1 and 2, GlucoMen day, Eversense, Eversense XL, S7 EasySense, Guardian, and Connect have been reported to be of great use to diabetics. Insulin pens are the most often utilized method of insulin administration in T2DM patients[155]. Users can track boluses, calculate remaining insulin, check insulin temperature, and receive dosage reminders using Bluetooth-enabled insulin pen caps and attachments that connect to smartphone apps[156]. The integration of insulin pumps with other diabetes technologies developed over the last decade has paved the way for techniques of optimally regulating blood glucose while minimizing user stress. For the management of LADA C-peptide levels should be monitored every 6 mo. For KPDM patients lifestyle modifications as stated

Table 1 List of various circulating microRNAs reported in diabetes mellitus individuals

Mechanism/pathway (diabetes type)	Expression of miRNAs	Ref.
Endothelial dysfunction (T2DM)	↑miR-28-3p	[87]
	↓miR-24	
	↓miR-21	
	↓miR-20b	
	↓miR-15a	
	↓miR-126	
	↓miR-191	
	↓miR-197	
	↓miR-223	
	↓miR-320	
	↓miR-486	
	↓miR-150	
	↓miR-29b	
	↓miR-107	
	↓miR-132	
	↓miR-144	
Glucose metabolism (T2DM)	↑miR-9	[88]
	↑miR-29a	
	↑miR-30d	
	↑miR-34a	
	↑miR-124a	
	↑miR-146a	
	↑miR-375	
Inflammation (T2DM)	↓miR-146a	[89]
Glucose metabolism (T2DM)	↑miR-27a	[90]
	↑miR-320a	
Glucose metabolism (T2DM)	↓miR-126	[91-93]
Inflammation (T2DM)	↓miR-103b	[94]
Inflammation (T2DM)	↓miR-126-3p	[95]
	↓miR-21-5p	
Inflammation (T2DM)	↓miR-126	[96]
Endothelial dysfunction (T2DM)	↓miR-126	[97]
	↓miR-26a	
Glucose metabolism (T2DM)	↓miR-21	[98]
Inflammation (T2DM)	↓miR-126-3p	[99]
Endothelial dysfunction (T2DM)	↓miR-24	[100]
Platelet reactivity (T2DM)	↓miR-223	[101]
	↓miR-26b	
	↓miR-126	
	↓miR-140	
Glucose metabolism (T2DM)	↑miR-375	[102]

	↑miR-9	
Glucose metabolism (T2DM)	↑miR-30a-5p	[103]
	↑miR-150	
	↓miR-103	
	↓miR-28-3p	
	↓miR-29a	
	↓miR-9	
	↓miR-15a	
	↓miR-126	
	↓miR-145	
	↓miR-375	
	↓miR-223	
	↓miR-133	
	↓miR-107	
Endothelial dysfunction (miR-126); hypoxia (miR-210) (T2DM)	↓miR-126	[104]
	↑miR-210	
Angiogenesis (T2DM)	↑miR-193b-3p	[105]
	↑let-7i-5p	
	↑miR-199a-3-5p	
	↑miR-26b-5p	
	↑miR-30b-5p	
	↑miR-374a-5p	
	↑miR-20a-3p	
	↑miR-26a-5p	
	↑miR-30c-5p	
	↓miR-409-3p	
	↓miR-95-3p	
Apoptosis (T1DM)	↑miR-21	[106,107]
	↓miR-23a-3p	[108]
	↓miR-23b-3p	
	↓miR-149-5p	
Inflammation (T1DM)	↑miR-101a	[109]
	↑miR-30b	
-cell dysfunction (T1DM)	↑miR-106b-5p	[110,111]
	↑miR-222-3p	
	↑miR-181a	
T-cell dysfunction (T1DM)	↑miR-26a	[112]
	↑miR-98	[113]
	↑miR-23b	
	↑miR-590-5p	
-cell lymphopoiesis (T1DM)	↑miR-34a	[114]
DNA damage checkpoint (T1DM)	↑miR-200	[115]
Apoptosis (T1DM)	↓miR-144	[116]

Autoimmune imbalance (T1DM)	↓miR-146a	[117]
MODY	↑miR-103	[118]
MODY	↑miR-224	
Glucose metabolism (GDM)	↑miR-222	[119]
	↑miR-98	[120]
	↑miR-518d	[121]
	↑miR-340	[122]
	↑miR-130b, miR148a	[123]
-cell dysfunction (GDM)	↑miR-33a-5p	[124]
	↑miR-330-3p	[125]
	↓miR-494	[126]
	↓miR-96	[127]
	↓miR-221	[128]

miRNAs: MicroRNAs; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; GDM: Gestational diabetes mellitus; MODY: Maturity-onset diabetes of young.

above have been proposed to successfully treat the disease.

In addition to the above-mentioned methods, the following steps can be taken to control blood sugar levels.

Physical activity

Physical exercise is positively associated with controlled hyperglycemia levels among T2DM patients. Moderate physical activity (walking, gardening, regular household chores) on a regular basis has been shown to be an effective method to reducing the long-term symptoms of diabetes[157]. In women with type 2 diabetes, yoga practice is more beneficial than the same course of aerobic exercise in enhancing sleep quality, hence, yoga activity can thus be recommended to these patients[158]. The identification of cytokines such as irisin, osteocalcin, and adiponectin has led to the assumption that they may be important hormonal mediators of exercise therapy for diabetes and metabolic illnesses, although the precise mechanism remains unknown[159-161].

Dietary changes

Strict adherence to a restricted diet combined with adequate physical exercise is strongly linked to a lower incidence of diabetes[162]. The incorporation of a Paleolithic diet (a diet rich in lean meat, fish, fruits, and vegetables) into the daily routine of diabetic patients resulted in a significant improvement in glucose management[163]. Foods that are naturally abundant in dietary fiber also contain a variety of chemicals that may help decrease glycemia. For example, bioactive proteins, polyphenolic compounds, and other phytochemicals[164]. Additionally, according to current research, meal timing and frequency, missing meals, and fasting are all linked to metabolic syndrome. Eating frequently and in the morning may help to prevent metabolic syndrome. Understanding the impact of dietary choices on health is just as important as understanding the impact of nutrients on health.

Stress

The bulk of T2DM and T1DM-related parameters, including the release of glucose (and lipids) in circulation, the development of inflammatory cytokines, and raised blood pressure, are heavily influenced by psychological stress[165]. The underlying mechanisms entail a complex neuroendocrine structure that includes both the central nervous system and the peripheral nervous system. In one study, when type 2 diabetes patients were subjected to acute stress during the postprandial period, significant increases in blood glucose levels were seen[166]. Treatment options, including stress management therapies, appear to be a promising approach for effectively preventing or reducing type 2 diabetes incidence.

Sleep patterns

Another modifiable lifestyle choice that has been shown to influence metabolic health and energy status is sleep. Sleeping pattern optimization is critical in the diabetes management[167]. According to a population-based study, short sleep (less than 5 h) or insomnia is related to an elevated risk of T2DM [168]. Poor sleep was linked to increased glycated hemoglobin (HbA1c) levels (> 7%) and insulin

Table 2 Studies on polygenic risk score for type 1 diabetes mellitus and type 2 diabetes mellitus				
Diabetes type	SNPs	AUC for PRS	Ethnicity	Ref.
T1DM	41	0.87	Caucasian	[140]
T1DM	30	0.88	Caucasian	[141]
T1DM + T2DM	99	0.89	Caucasian	
T1DM	32	0.86	Caucasian	[142]
T1DM	32	0.90	Caucasian Hispanic	
T1DM	32	0.75	African-American	
T1DM	32	0.92	Asian-American	
T1DM	67	0.93	Caucasian	[143]
T2DM	3	0.58	Caucasian	[144]
T2DM	18	0.80	Caucasian	[136]
T2DM	16	0.75	Caucasian	[134]
T2DM	18	0.91	Caucasian	[135]
T2DM	22	0.74	Caucasian	[145]
T2DM	62	0.91	Caucasian United States population	[146]
T2DM	1000	0.79	Caucasian	[147]
T2DM	4	0.67	African	[148]
T2DM	7 million	0.73	Caucasian	[149]

SNP: Single nucleotide polymorphisms; AUC: Area under the curve; PRS: Polygenic risk score; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.

Table 3 Polygenic risk scores studies for gestational diabetes mellitus			
Diabetes type	SNPs	OR 95%CI	Ref.
GDM	34 SNPs previously associated with T2DM	1.11 (1.08-1.14)	[150]
GDM	11 SNPs previously associated with T2DM	1.18 (1.10-1.27)	[151]
GDM	150 previously associated with T2DM	1.06 (1.01-1.10)	[152]
GDM	84 SNPs	6.15 (5.03-7.51) top 5%	[153]

SNP: Single nucleotide polymorphisms; OR: Odds ratio; T2DM: Type 2 diabetes mellitus; GDM: Gestational diabetes mellitus.

resistance in T2DM patients in previous research[167]. Similar results has been observed for T1DM also, where persons with T1DM who reported sleeping more than 6 h had 0.24% lower A1C values than those who slept less than 6 h[169].

One-step or two-step diagnosis for GDM

The one step or two step techniques are used to diagnose gestational DM. The one step method consists of a 2-h oral glucose tolerance test with a 75-g glucose overload that examines plasma glucose concentration at fasting, 1 h, and 2 h following glucose delivery. A positive result is characterized as a number more than 92, 180, or 153 mg/dL[170-172]. The two-step method comprises a nonfasting oral 50-g glucose load followed by a glucose blood measurement 1 h later. A positive result is defined as a blood glucose level greater than 130, 135, or 140 mg/dL; the most used number is 135 mg/dL. A diagnostic test is performed after a positive screening test[173].

PHARMACOGENOMICS IN DIABETES MELLITUS

Pharmacogenomics is the process of developing a genetically personalized therapy strategy to obtain

the best optimal individual response. Several polymorphisms in the genes *i.e.*, *ABCC8*, *KCNJ11*, *TCF7L2*, *CYP2C9*, *IRS1*, *CDKAL1*, *CDKN2A*, *CDKN2B*, *KCNQ1*, *NOS1AP*, and *CAPN10* have been explored in recent years in relation to the therapeutic response of various anti-diabetic medicines[174]. The American Association of Clinical Endocrinologists/American College of Endocrinology and the ADA in addition to metformin had proposed four oral options (sulfonylurea, thiazolidinedione, dipeptidyl peptidase-4 inhibitor, sodium-glucose cotransporter 2 inhibitor) and injectable agents (glucagon-like peptide-1 receptor agonist or basal insulin) for lowering blood glucose levels (Figure 3). Although these drugs have important therapeutic effects on diabetes, their long-term impact has not been accomplished, and their responses in individuals also display variances[175,176]. Moreover, some agents produce adverse side effects, such as hypoglycemia, weight gain, gastrointestinal discomfort, urogenital infections, discomfort at the injection site, and in some cases heart failure[177].

Potential therapeutic drugs with new targets for diabetes

It is important to identify and develop novel targets to improve the therapeutic efficacy of present anti-diabetic medications, reduce the risk of side effects, and even reverse the development of diabetes. Many potential antidiabetic drugs *i.e.*, Dorzagliatin (glucokinase activators), BI 135585 [b-hydroxysteroid dehydrogenase-1 inhibitors (11-b-HSD1 inhibitors)], DS-8500a (G-protein-coupled receptor 119 agonists), and PF-06291874/LGD-6972 (glucagon receptor antagonists) with new targets are currently undergoing clinical trials. These drugs may become new diabetes treatment options and provide more therapeutic alternatives for diabetes patients.

There is growing evidence that vitamin D insufficiency may play a critical role in the T2DM etiology [178]. Thus, in a randomized controlled study, the oral daily doses of vitamin D supplementation with metformin significantly reduced HbA1c levels after 3 and 6 mo of supplementation, compared to the metformin alone[179].

PHYTOCONSTITUENTS: AN ALTERNATIVE OPTION

In diabetic patients, monotherapies combined with herbal extracts or phytoconstituents demonstrated significant improvements in blood glucose levels. Plant-derived chemical compounds have also proven to be potential alternatives. Table 4[180-194] shows the known effects of various phytoconstituents on diabetes. Diabetes can be managed using either nonpharmacological (reasonable diet and exercise) or pharmacological (drugs or insulin) techniques. However, T2DM medication is expensive for patients and has substantial adverse effects. Plants appear to offer an appealing alternative to traditional diabetes treatment. They comprise complex compounds including many natural bioactive principles with less adverse effects.

CONCLUSION

Diabetes pathogenesis encompasses genetic, epigenetic, and environmental variables and their interactions. To date, the examined common variations can explain just a small portion of the heritability of diabetes. Furthermore, the technique of integrating the associated variants as a type of GRS does not accurately predict diabetes risk. As a result, the trend for genetic risk factors for diabetes is shifting from common to rare variants. Aside from genetic variables, systemic data from other trans-omics such as epigenomics, transcriptomics, proteomics, metabolomics, and metagenomics will contribute to a better understanding of genetic determinants in the progression of metabolic illnesses like diabetes. Technological, computational, and collaborative developments continue to uncover novel genetic diabetes risk factors. There are high prospects for tailored diabetes treatment in the future, based on increased knowledge of the molecular genetic profile of the patients.

Table 4 List of phytochemicals used in the prevention and treatment of diabetes and its complications

Phytochemical	Source	Outcomes	Ref.
Curcumin	Curcuma longa	↑Insulin sensitivity, ↓blood glucose levels, and hypoglycemia	[180]
Rutin	Buckwheat (Fagopyrum esculentum)	↓Hepatic glucose production, ↑glucose tolerance	[181]
Resveratrol	Grapes, plums, peanuts, nuts, red wine	Improved insulin signaling, ↑glucose-mediated insulin secretion	[182]

Quercetin	Apples, black tea, berries, capers, red wine, onions	↑Glucose uptake, ↓hepatic glucose production	[182, 183]
Genistein	Legumes	Improved lipid glucose metabolism and ↓fasting glucose	[184]
Hesperidin	Orange, lemon	↑Glucose uptake, ↓HbA1c, ↓oxidative stress	[185]
Naringin	Skin of grapefruit and orange	↓Hepatic glucose production, ↓oxidative stress, ↑glucose uptake	[185]
Naringenin	Citrus fruits, tomatoes, cherries, grapefruit, cocoa	↑Glucose uptake, ↓glucose intolerance and reduced blood glucose levels	[186]
Vitamin A, D, and E	Eggs, yellow, red, and green (leafy) vegetables, such as spinach, carrots, sweet potatoes and red peppers. yellow fruit, such as mango, papaya and apricots	↓Glucose intolerance, ↓hyperglycemia	[182]
Fisetin	Strawberry, apple, persimmon, grape, onion, and cucumber	↓Hepatic glucose and ↑glucose metabolism	[187]
Flavonoids	Coffee, guava tea, whortleberry, olive oil, propolis, chocolate, and cocoa	↓Glucose absorption, inhibition of advanced glycation end products	[188]
Isoflavones	Soybean	Improves glucose metabolism	[189]
Catechins	Tea leaves and red wine	Promote insulin sensitivity	[190]
Hydroxycinnamic acids	Fruits and vegetables, especially the outer part of ripe fruits	Promote glucokinase activity	[191]
Caffeoylquinic	Potatoes, eggplants, peaches, prunes, and coffee beans	Promote insulin response	[192]
Anthocyanins and anthocyanidins	Berries, eggplants, avocado, oranges, olives, red onion, fig, sweet potato, mango, and purple corn	Promote blood glucose regulation	[193]
Stillbenoids	Grapevine, berries, and peanuts	Promote pancreatic -cell and hepatoprotective activity	[194]

HbA1c: Glycated hemoglobin.

FOOTNOTES

Author contributions: Goyal S, Rani J, Bhat MA, Vanita V contributed data collection; Goyal S, Rani J, Bhat MA, Vanita V contributed manuscript writing; Vanita V contributed to edit the manuscript; All the authors have read and approved the final version of this manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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Country/Territory of origin: India

ORCID number: Shiwali Goyal 0000-0002-3126-774X; Jyoti Rani 0000-0002-3375-3266; Mohd Akbar Bhat 0000-0002-2637-9157; Vanita Vanita 0000-0003-2251-6641.

S-Editor: Li L
L-Editor: A
P-Editor: Guo X

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What's old is new again: Insights into diabetic foot microbiome

Azza A H Rajab, Wael A H Hegazy

Azza A H Rajab, Wael A H Hegazy, Department of Microbiology and Immunology, Faculty of Pharmacy, Zagazig University, Zagzig 44511, Egypt

Corresponding author: Wael A H Hegazy, PhD, Full Professor, Department of Microbiology and Immunology, Faculty of Pharmacy, Zagazig University, Zagzig 44511, Egypt. waelmhegazy@daad-alumni.de

Abstract

Diabetes is a chronic disease that is considered one of the most stubborn global health problems that continues to defy the efforts of scientists and physicians. The prevalence of diabetes in the global population continues to grow to alarming levels year after year, causing an increase in the incidence of diabetes complications and health care costs all over the world. One major complication of diabetes is the high susceptibility to infections especially in the lower limbs due to the immunocompromised state of diabetic patients, which is considered a definitive factor in all cases. Diabetic foot infections continue to be one of the most common infections in diabetic patients that are associated with a high risk of serious complications such as bone infection, limb amputations, and life-threatening systemic infections. In this review, we discussed the circumstances associated with the high risk of infection in diabetic patients as well as some of the most commonly isolated pathogens from diabetic foot infections and the related virulence behavior. In addition, we shed light on the different treatment strategies that aim at eradicating the infection.

Key Words: Diabetic foot infection; Chronic ulcer; Bacterial biofilm; Multidrug resistance; Methicillin resistant *Staphylococcus aureus*; Vancomycin resistance

Core Tip: Diabetic foot infection is a common complication of diabetes that can lead to serious consequences, such as amputations and even death. The microbiome of the wound plays a crucial role in the development and progression of diabetic foot ulcer. The current review shed light on the most prevalent bacterial infections and their related virulence factors that are associated with diabetic foot complications. Additionally, various approaches for treatment were explored.

INTRODUCTION

Diabetes is a chronic metabolic disorder that is characterized by the failure of the body to regulate blood glucose levels. The worldwide prevalence of diabetes has increased to epidemic levels in the last decade; the latest report from the International Diabetes Federation Diabetes Atlas stated a global diabetes prevalence of 10.5% in 2021 with the expected incidence to reach 12.2% in 2045. By comparing to the 2019 report, which stated a 9.3% global incidence of diabetes with a 2045 rate projection of 10.9%, the data suggest an exaggerated increase in diabetes prevalence worldwide[1,2]. Diabetes is associated with many complications that are commonly encountered in health care facilities, especially cardiovascular disease, retinopathy, neuropathy, nephropathy, and lower limb infections in addition to the high risk of amputations and systemic infections that are linked to high mortality rate[3,4]. Diabetic foot ulcer is a serious condition characterized by chronic lower limb wound that is often complicated by disseminating polymicrobial infections that can affect the underlying bone tissues. Diabetic foot infection (DFIs) require careful attention from health care providers regarding the proper diagnosis of the wound level and prompt management including debridement procedures, antimicrobial treatments, and follow-up of the wound healing process[5-7].

During the examination of the diabetic foot wound, the accurate evaluation of the wound plays a pivotal role in the proper management selection. Usually, the wound examination should include specimen collection from the deepest parts of the wound in order to identify the associated etiologic pathogens, accompanied by inspection of the underlying vascular and bone tissues. The Meggitt-Wagner guide is a commonly used system for classification of the DFI based on three parameters: the depth of the ulcer; the infection level; and the degree of necrosis. The guide classifies the DFI into five main categories, which are outlined in Figure 1. A progressive DFI needs immediate management in order to minimize the risk of bone infection and osteomyelitis, which are common complications in 50%-60% of severe infections and associated with a high risk of limb amputations[8,9]. In this review, we discussed the most common pathogens related to DFIs along with the associated virulence factors and possible treatment options for eradication of the infection and subsequent minimization of comorbidities and mortality rates.

FACTORS THAT INCREASE THE RISK OF INFECTION IN DIABETIC PATIENTS

Impaired immunity

Impaired immune functions represent a defining element in diabetes that impacts both innate and adaptive immunity. The innate immunity is the first line defense against pathogens and foreign particles. The response is mediated through phagocytes, natural killer cells, and inflammation[10]. Diabetes is associated with elevated levels of tumor necrosis factor α, macrophages, and inflammatory cytokine release that predisposes patients to chronic inflammation and increased pathogenicity of infections[11]. Additionally, diabetes is associated with an impaired number and functioning of natural killer cells with high connectivity to autoimmune diseases and increased risk of cardiovascular disease, malignancy, and susceptibility to infection[12]. On the other hand, the decreased number and function of dendritic cells results in impaired antigen presenting function and subsequently deterioration of the function of adaptive immunity[12]. Likewise, diabetes is associated with marked suppression in release of interleukin 6, decreased antibody production, decreased effector T cell development, and impaired leukocyte recruitment, all of which are considered important mediators of the adaptive immune response against pathogens[10,13].

Hyperglycemia

Elevated blood glucose level is the main symptom of diabetes; failing to control blood glucose levels in diabetic patients will cause serious complications as a result of alterations in multiple metabolic pathways[14]. The high blood glucose level results in activation of the polyol pathway, increased glycation of end products, and eventually boosted release of reactive oxygen species and nitric oxide that contribute to oxidative stress and inflammation[15]. Hyperglycemia also contributes to immuno-suppression through inhibition of cytokine release in response to pathogenic infection in addition to attenuation of macrophages, neutrophil dysfunction, and complement activation[10,13]. In addition, hyperglycemia is associated with stiffer blood vessels, which cause slower circulation and capillary dysfunction, predisposing to reduced tissue oxygenation[16]. Moreover, hyperglycemia contributes to
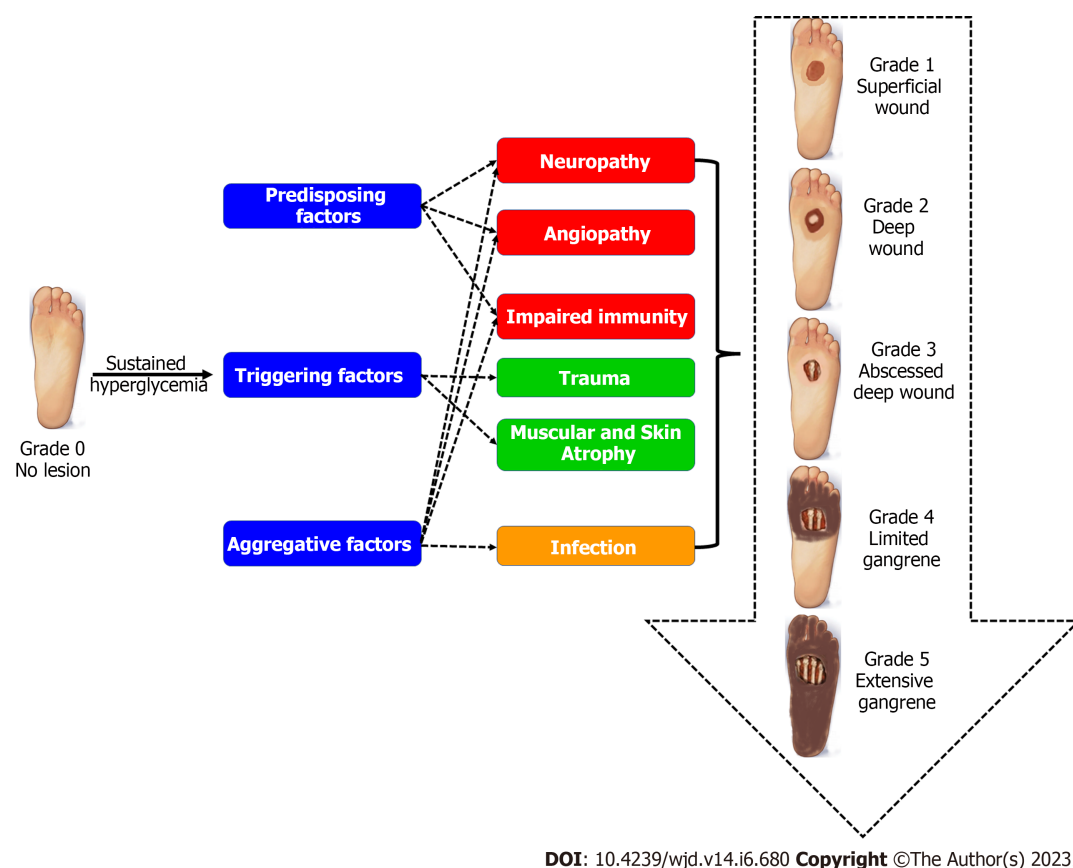
Figure 1 Risk factors for the development of diabetic foot infections. Angiopathy and neuropathy are the main predisposing factors of diabetic foot infections (DFIs), together with muscular atrophy and extrinsic triggers, such as trauma, in the presence of abnormal immunity and ischemia as aggravating factors. These factors collectively result in the loss of skin integrity favoring the development of DFIs. The Meggitt-Wagner classification is commonly used to grade the DFIs (from 1 to 5) on three characteristics: the depth of ulcer; the degree of infection; and the necrosis.

increased virulence of some pathogens as observed in some coronavirus disease 2019 patients with type 2 diabetes mellitus where an uncontrolled blood glucose level was directly linked to increased severe acute respiratory syndrome coronavirus 2 replication and increased severity of complications[17]. This is in accordance with multiple studies that confirmed the association of hyperglycemia with increased bacterial load and virulence expression in *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) infections accompanied by increased severity of the infection in diabetic patients[18,19].

Vasculopathy and ischemia

As mentioned earlier, persistent hyperglycemia results in overproduction of reactive oxygen species and superoxides especially peroxynitrite leading to increased nitrosylation and eventually causing endothelial dysfunction, vasoconstriction, and platelet aggregation. In addition, the diabetic proinflammatory environment results in vascular inflammation and proliferation of vascular smooth muscles predisposing to atherosclerosis and atherothrombosis[20]. Some of the common vasculopathy presentations in diabetic patients involve peripheral artery diseases giving way to peripheral cramps, numbness, discoloration of limbs, weak pulse in the affected limb, and critical limb ischemia[21]. Peripheral ischemia results in delayed wound healing and tissue necrosis as a result of a decreased supply of oxygen, nutrients, and immune cells; in addition, the reduced tissue perfusion would limit the delivery of antibodies and antibiotics. A combination of all the preceding factors would result in an environment that favors microbial proliferation at the injured tissues, which supports the development of chronic diabetic foot ulcers[22].

Neuropathy

Diabetic neuropathy is a neurodegenerative disorder that affects the peripheral sensory nervous system in 50% of cases. The condition is characterized by pain, numbness, and loss of sensory function that begins in the lower extremities[23]. Again, hyperglycemia along with the associated inflammation and oxidative stress play the lead role in the mechanisms predisposing to diabetic neuropathy, where Schwan cells and the myelin sheath are the first affected resulting in delayed signal transmission and eventually neuron dysfunction especially in distal terminals of motor nerve axons[23,24]. Diabetic neuropathy contributes to increased risk of infection in diabetic patients through inhibition of local

vasodilation of the microcirculation at the affected tissues, which is a normal response to injury or inflammation; the reduced vasodilation results in reduced local blood flow and further promotes local ischemia[25]. On top of that, the loss of sensory nervous function will impair pain sensation, thus diminishing the ability of the patient to sense or detect wounds and injuries in peripheral tissues especially toes and foot soles, which in turn leads to delayed response and management of the condition and increasing the risk of amputation[26]. Peripheral neuropathy is a common manifestation in 90% of hospital admissions of diabetic foot ulcers; in addition, 14%–24% of people with a diabetic foot ulcer will ultimately undergo an amputation procedure with subsequent high mortality rate[24].

BACTERIAL VIRULENCE FACTORS AND THEIR CONTRIBUTION TO PATHOGENICITY IN DFIS

Adhesins
Adhesins are fine protein extensions expressed on the bacterial cell surface usually represented by a small protein subunit at the tip of the fimbriae. Their primary function is to facilitate the attachment or adherence of bacteria to host cells, which is the first step in initiation of an infection[27,28]. Adhesins also play a pivotal role in establishment of biofilms. This fact was proven by many studies that reported that biofilm formation can be completely blocked by downregulation of pili expression or by using adhesins antibodies that can drastically inhibit bacterial attachment to the target tissues, hence inhibiting subsequent initiation of infection and biofilm formation[29,30]. Some adhesins are called hemaglutinins due to their ability to induce the agglutination and hemolysis of red blood cells. Hemaglutinins contribute to localized destruction of red blood cell (RBCs) and release of iron, which is an essential nutrient requirement for most pathogenic bacteria[31]. Additionally, bacterial adhesins play an important role in intracellular bone invasion as observed in the ability of *S. aureus* to invade osteoblasts and fibroblasts, which contribute to serious complications of diabetic foot ulcer as well as increased risk of amputation[32].

Biofilm formation
Biofilm formation represents an important virulence factor that plays a leading role in the persistence and recurrence of diabetic foot ulcers. Biofilms are closed microbial communities embedded in a mucoid extracellular polymer matrix consisting of a wide range of molecules including polysaccharides, proteins, glycoproteins, glycolipids, cell debris, wastes, and surfactants[33,34]. These molecules provide high viscosity to the biofilm matrix acting as a physical protective barrier that prevents penetration of host immune defenses as well as antimicrobial treatments[35]. In addition, diabetic patients suffer from reduced peripheral blood supply, which makes the it even harder for the immune system and antibiotic treatments to eradicate biofilms in DFIs[36].

Within the biofilm, bacteria can coordinate their behavior using a communication system called quorum sensing (QS). This system is activated once the bacterial population reaches a certain threshold level beyond which the members of the biofilm initiate a coordinated group response that favors the public interests of the biofilm community; this coordinated activity aims at conserving energy and nutrients by reducing the metabolic activity of biofilm inhabitants[37-39]. Additionally, bacterial gene expression is directed towards increased expression of virulence factors especially extracellular toxins, which initiate extensive tissue destruction at the biofilm site; this ensures generous release of nutrients from the damaged tissues as well as facilitating the spread of infection to adjacent tissues, which further cements the biofilm and increases its persistence[40,41].

Another important feature of biofilms is the shift in bacterial phenotypes within the biofilm community towards the formation of persister cells that are inherently resistant to eradication by antimicrobial agents. Persister cells are dormant slow-growing cells with altered metabolic pathways that result in loss of the target site of most antibiotic treatments hence contributing to persistence and recurrence of biofilm ulcers[42]. At the same time, the high bacterial population within the biofilm results in an increased rate of horizontal gene transfer (HGT) between biofilm inhabitants, creating a rich pool of characteristics that eventually lead to natural selection of virulence genes and antimicrobial resistance genes[43]. Indeed, it was reported by many studies that biofilm formation is highly linked to an increased rate of antimicrobial resistance in DFIs, which contributes to a high incidence of chronic recurrent ulcers and higher risk of amputations[36,44].

Tissue damaging exoenzymes
Enzymes like proteases, collagenase, hyaluronidase, lipases, fibrinolysin, gelatinase, and elastase are all upregulated in diabetic foot biofilms under control of QS[45-48]. Such enzymes play an important role in inducing tissue damage, which helps in the release of nutrients that are required by the pathogens for growth[49-51]. Additionally, the vascular tissue damage would diminish tissue perfusion and contribute to the reduced ability of the immune system and antibiotic treatments to reach the site of the infection[52]. At the same time, the destroyed physical integrity of the tissues facilitate invasion of

adjacent tissues and spread of the infection. Moreover, proteases result in delayed healing of the affected tissues, which further contributes to the chronic nature of diabetic foot ulcers[16,49]. Immuno-globulin proteases represent a different category of proteases that target humoral components of immune defense (mainly immunoglobulin A, immunoglobulin M, and immunoglobulin G) rather than inducing generalized tissue damage[53,54]. Immunoglobulin proteases represent an important virulence factor in many pathogens that allows them to evade the host immune response[55,56]. Local therapy with protease inhibitors is an essential element in control of diabetic foot ulcer in order to improve wound healing and minimize the complications accompanying chronic wounds[49,57].

Hemolysins and leukocidins

Hemolysins and leukocidins belong to a group of pore-forming toxins that destroy blood cells by inducing perforation in the cell membrane and subsequent cell lysis[58-60]. Hemolysins are important virulence factors in pathogenic infections since they induce RBC lysis and release of iron, which is an essential nutrient requirement for pathogens. Iron is an important element for life since it is required for making important enzymes in all living cells[36,59,61,62]. However, iron is never found in a free form in biological tissues or in the extracellular fluids; the ability of most pathogens to survive in an iron-free environment highly depends on its iron acquisition talents including hemolysin and siderophore production[63].

S. aureus is one of the most common causative agents of DFIs. *S. aureus* is equipped with an arsenal of toxins including four hemolysins targeting a wide range of host cells: α-hemolysin (mainly targeting lymphocytes and monocytes); β-hemolysin (targeting human monocytes and sheep erythrocytes with no effect on human erythrocytes); γ-hemolysin (highly toxic to neutrophils); and δ-hemolysin (toxic to erythrocytes). The combined actions of these toxins result in RBC hemolysis as well as inhibition of leukocyte function and subsequent evasion of host immune defenses[64,65].

Antimicrobial resistance

Antimicrobial resistance is an escalating worldwide problem with increased prevalence among diabetic patients. As discussed previously, diabetic patients are at high risk of contracting microbial infections especially due to their immunocompromised status, which leads to higher rates of persistent difficult to treat infections, and such circumstances usually predispose to higher probability of development of antimicrobial resistance[66-68]. This relationship can be explained based on many factors: (1) The development of bacterial biofilms in chronic infections, like in cases of diabetic foot ulcers, is associated with activation of QS communication systems, which in turn induces upregulation of virulence gene expression including antimicrobial resistance genes[69-71]; (2) Bacterial biofilms are also associated with an increased rate of HGT between members of the biofilm community, which means increased rate of transfer of antibiotic resistance genes between different species within polymicrobial biofilm communities[72]; and (3) Chronic infections are usually associated with prolonged antimicrobial treatment courses, especially with broad spectrum antibiotics that exert stress pressure on pathogenic bacteria leading to natural selection of resistant strains[73,74].

Similarly, antibiotic self-administration and empirical prescription of broad-spectrum antibiotics by general practitioners are considered predisposing factors for higher rates of development of antibiotic resistance in diabetic patients[75-77]. One interesting observation was discussed in a previous study that reported a three-fold higher incidence of antibiotic resistance in diabetic foot patients in 2020 as compared with individuals admitted with the same diagnosis in 2019. The authors linked this observation to the circumstances that surrounded the coronavirus disease 2019 pandemic with increased administration of antibiotics for control of the infection complications, bearing in mind the fact that diabetic patients were among the high-risk categories at that point[78,79].

Additionally, some diabetic foot ulcers can result from impaired healing of wound tissues rather than the presence of wound infection. Therefore, it is highly recommended to avoid empirical antibiotic treatments before confirming the presence of an infection in diabetic foot ulcers. In addition, antibiotic therapy should not be given for uninfected foot wounds as prophylaxis against infection or as a method to improve wound healing[50,80]. Instead, it is advised to collect wound samples or swabs for microbio-logical examination in order to confirm the presence or absence of infection. This also allows for identi-fication of the causative pathogen in cases of confirmed infection as well as performing antimicrobial susceptibility testing in order to identify the optimum antimicrobial treatment for every individual case [81,82].

On a similar basis, the administration of broad-spectrum antibiotics for recurrent episodes of diabetic foot ulcers is not required, as recommended by a recent study that concluded that a patient history of previous DFIs does not necessarily reflect a higher risk of antibiotic resistance in subsequent episodes [83]. Boschetti *et al*[84] documented the resistance patterns of the most prevalent bacterial species isolated from DFIs to different classes of antibiotics when administered as a monotherapy or as a combination treatment. The results presented in Figure 2 provide an alarming outlook at the dangerous growing levels of antimicrobial resistance in many antimicrobial groups[84].
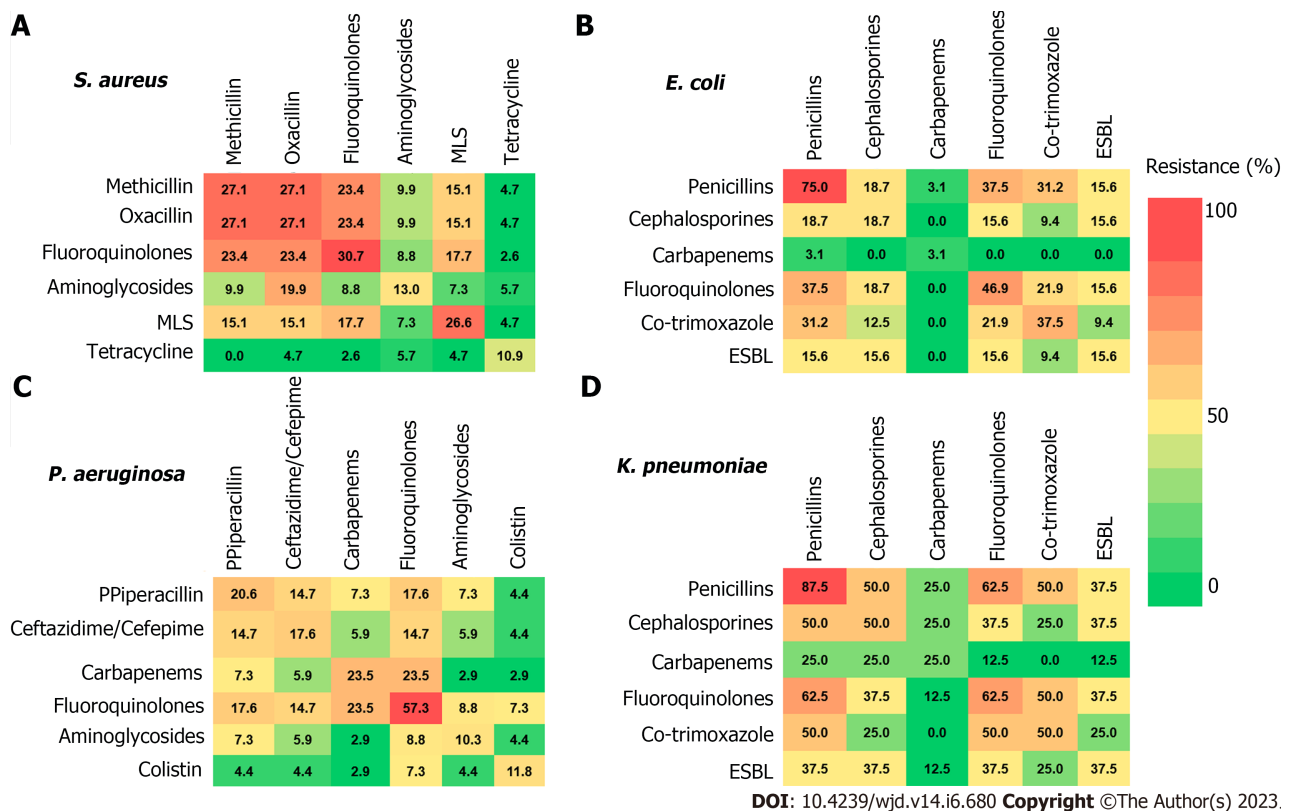
Figure 2 Resistance of bacteria isolated from diabetic foot infections to different classes of antibiotics as monotherapy or in combinations. A: *Staphylococcus aureus* (*S. aureus*); B: *Escherichia coli* (*E. coli*); C: *Pseudomonas aeruginosa* (*P. aeruginosa*); D: *Klebsiella pneumonia* (*K. pneumonia*). The data presented as percentages of resistance, adopted from Boschetti *et al*[84]. Resistance to oxacillin expects resistance to cephalosporines, carbapenems, and β-lactams. MLS: Macrolides, lincosamides, and streptogramines; ESBL: Extended spectrum beta-lactamases.

THE MOST PREVALENT BACTERIAL DFIS

The dwindled immunity of the diabetic patients paves the way for easy contraction of opportunistic pathogens from the patient's environment, leading to high risk of the progression of minor foot injuries into life-threatening infections[85,86]. The Meggit-Wagner system is the most commonly used classification guide of DFIs that assesses the ulcer depth, the presence of osteomyelitis, and/or gangrene using an ascending level from 0 to 5[87,88]. The more aggressive pathogenic bacterial infections are usually denoted by a higher level number[85,89,90]. There are multiple variables contributing to the establishment and progression of the infection, mainly: (1) Host response; (2) Ulcer location; (3) Tissue perfusion; and (4) Ulcer depth[87,91,92]. Upon trying to identify the etiologic agents behind DFIs, it is hard to name one exclusive pathogenic agent since DFIs are always caused by polymicrobial infections [90,93,94].

It is noteworthy that the polybacterial nature of DFIs makes the identification of different bacterial species a difficult task and mandates the application of both phenotypic and genotypic detection methods[91,95]. Several studies documented that the most prevalent bacterial species isolated from DFIs are *S. aureus*, *Escherichia coli* (*E. coli*), *P. aeruginosa*, *Proteus* spp., *Klebsiella* spp., and *Enterococcus* spp. with variable prevalence rates that are presented in Figure 3[32,96]. The following section shed light on the most prevalent Gram-negative and Gram-positive bacterial DFIs especially those isolated from deep wounds with higher Wagner grades.

Staphylococcus spp.

Staphylococcus spp. are Gram-positive cocci that are ubiquitous in the environment. They are divided into pathogenic *S. aureus* and opportunistic coagulase negative *Staphylococcus* spp.[97-99]. However, the coagulase negative *Staphylococcus* spp. (*S. epidermidis*, *S. saprophyticus*, and others) are prevalent in the normal skin flora and could cause aggressive opportunistic infections in diabetic foot wounds[97,100]. *S. aureus* is considered by far the most commonly isolated species from macerated DFI especially in wounds of higher Wagner grade. It accounts for 20%-25% of all isolated bacteria[86,88-90,92]. The predominance of *S. aureus* in diabetic foot wounds can be attributed to: (1) Their ubiquitous presence in the environment; (2) The high ability of *S. aureus* to survive and resist bactericidal agents especially in healthcare settings giving rise to nosocomial infections; (3) A robust arsenal of virulence factors that facilitates anchoring of *S. aureus* infection; (4) The significantly high biofilm forming ability of *S. aureus*;
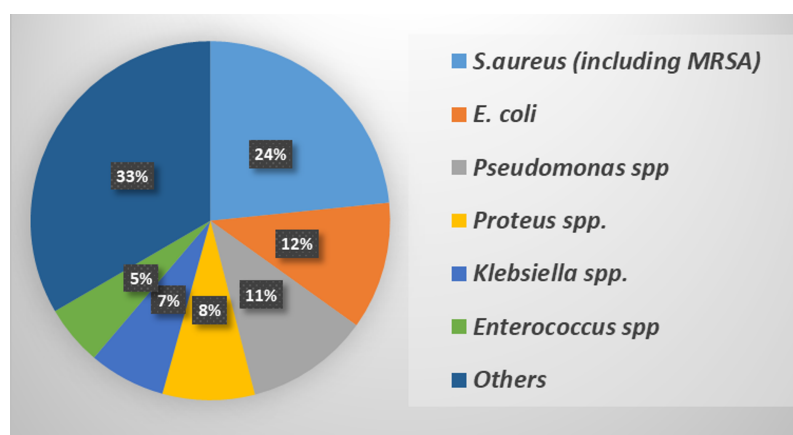
DOI: 10.4239/wjd.v14.i6.680 **Copyright** ©The Author(s) 2023.

Figure 3 Frequency of isolated bacterial species from diabetic foot infections. The presented data were collected from 57 studies that represented 6736 clinical samples, yielding 8418 microbial isolates[96]. *S. aureus*: *Staphylococcus aureus*; *E. coli*: *Escherichia coli*; MRSA: Methicillin-resistant *Staphylococcus aureus*.

and (5) The especially high rate of HGT between *S. aureus* and other members of a polymicrobial population leading to an increased ability of *S. aureus* to gain antibiotic resistant genes[85,86,88,90,91,94, 95,101-103]. *S. aureus* has a collection of different virulence factors including the production of diverse extracellular enzymes such as coagulase, gelatinase, hemolysins, and proteases in addition to a cocktail of toxins, such as pore-forming toxins, α-toxin, exfoliative toxin, enterotoxin, toxic shock syndrome toxin, and the virulent pigment staphylolysin[32,95,97,98].

The recent increase in the rates of antibiotic resistance patterns requires careful attention during the choice of a proper antimicrobial treatment. Methicillin-resistant *S. aureus* (MRSA) is a problematic pathogen that continues to grow as a public health concern[95,101,102]. Unfortunately, several studies have reported an increased rate of MRSA in polymicrobial DFIs as demonstrated in Figure 4[85,88,94,95, 102-135]. Although the complete identification of the full bacterial spectrum in a DFI is sometimes difficult, the detection of MRSA can be easily confirmed using the Kirby-Bauer antibiotic disk method in addition to genotypic detection methods[91,95]. Generally, vancomycin has been and still is the pillar therapy for MRSA. However, there is a growing mass of evidence that the minimum inhibitory concentrations of vancomycin to MRSA are increasing globally[106].

E. coli

E. coli is one of the most common causative pathogens of DFIs with a high incidence of biofilm formation[96] *E. coli* is also considered one of the most common causes of Gram-negative bacteremia in hospitalized patients[34,136]. *E. coli* is an opportunistic pathogen that is a common member of the human skin and colon flora[137]. The initiation of a pathogenic lifestyle in *E. coli* infection benefits from multiple virulence factors that allow for colonization and tissue destruction at different body organs especially in immunocompromised individuals. *E. coli* adhesins, mainly type 1 fimbriae and P fimbriae, are important virulence factors that are essential for adhesion and initiation of the infection[138,139]. Additionally, adhesins play an important role in diabetic foot pathogenesis due to their role in cytokine induction, tissue inflammation, and biofilm initiation[138]. *E. coli* also secretes hemolysin and siderophores that induce RBC damage and subsequent iron acquisition from the damaged tissues[140]. Importantly, many studies have confirmed a positive correlation between the hemolytic activity, biofilm formation, and high levels of antimicrobial resistance in *E. coli* infections[141,142].

P. aeruginosa

P. aeruginosa is a Gram-negative bacillus that is characterized with an armory of virulence factors including multiple bacterial surface structures such as pili and flagella in addition to a diverse array of extracellular toxins[143-145]. The observed prevalence of *P. aeruginosa* in DFIs is fluctuating from high to moderate levels, yet it is still among the most prevalent bacterial infections in DFIs[83,86,90,93,96,107, 125,131,146-149]. *P. aeruginosa* employs five secretion systems (T1SS, T2SS, T3SS, T5SS, and T6SS) that are used to regulate bacterial survival and utilized in establishment of infection[143,145]. Additionally, *P. aeruginosa* has at least three types of QS communication systems that orchestrate the expression of several virulence factors such as biofilm formation, motility, resistance to host immunity, and production of extracellular toxins such as protease, lipase, hemolysin, elastase, and pyocyanin pigments [150].

Furthermore, *P. aeruginosa* has a remarkable ability to acquire antibiotic resistance against most of the commonly used antibiotics, making its eradication a difficult task[143,146,149]. *P. aeruginosa* can easily
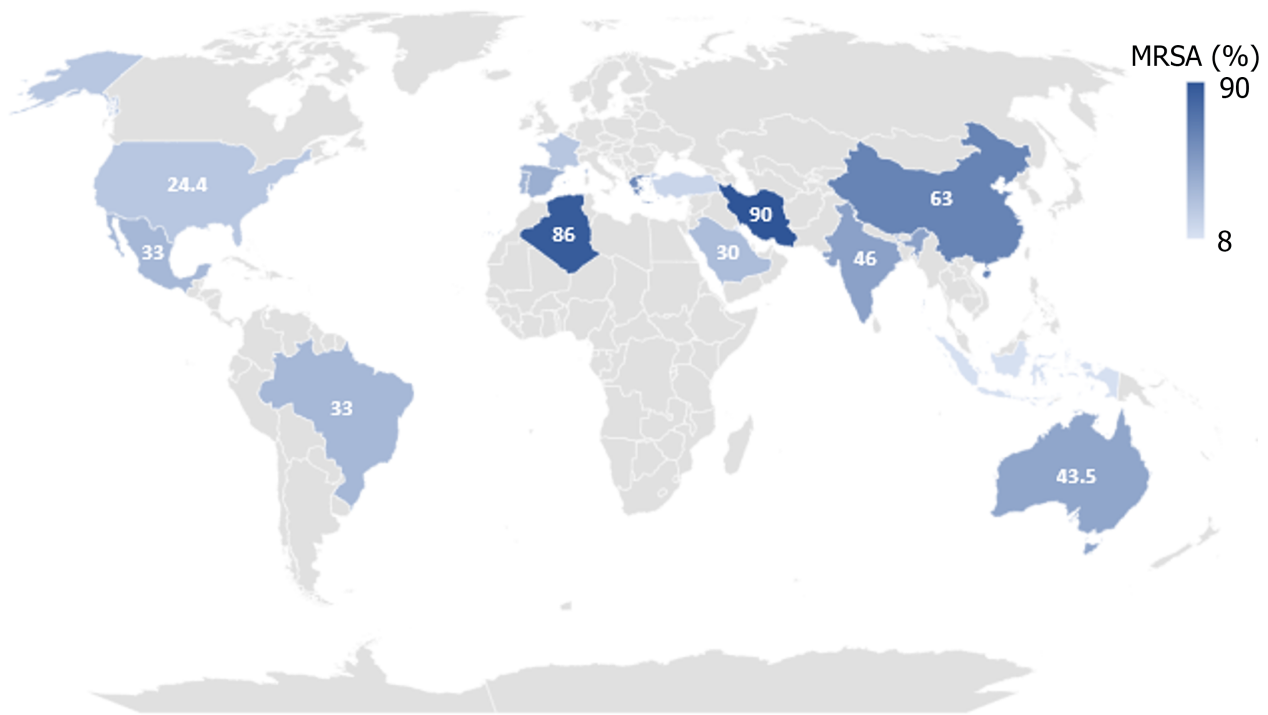
Figure 4 Prevalence of methicillin resistant *Staphylococcus aureus* isolated from diabetic foot infections around the world. The presented data are percentage of methicillin resistant *Staphylococcus aureus* (*S. aureus*) from the isolated *S. aureus* from diabetic foot infections.

establish an infection on intact healthy skin[147,148] and even more so on already vulnerable tissues in immunocompromised patients such as in diabetic foot wounds[146,148,149]. The guidelines provided by the American Infectious Diseases Society for DFIs state that empiric therapy directed against *P. aeruginosa* is usually not recommended[147,149]. However, once the infection is identified, it is recommended to perform antibiotic susceptibility tests of the bacterial isolates[151-153]. There are several classes of antibiotics that are proposed as a monotherapy or as a combination therapy for eradication of *P. aeruginosa* in DFIs, including fluroquinolones, aminoglycosides, and colistin[83,84,143, 147,151,152].

Proteus mirabilis

Proteus mirabilis (*P. mirabilis*) is a Gram-negative bacterium that is famous for its swarming motility and its remarkable survival in challenging environmental conditions[154,155]. The ability of *P. mirabilis* to initiate a pathogenic infection depends on multiple virulence factors such as multiple types of fimbriae and adhesins that allow attachment to different surfaces, giving rise to the remarkable stickiness and biofilm-forming ability of the bacterium onto many surfaces and at different conditions[156]. Additionally, *P. mirabilis* secretes a lethal cocktail of extracellular toxins including proteases, hemolysin, and urease, which all contribute to the extensive tissue damage and inflammation at the infection site [157]. Another significant feature of *P. mirabilis* is the formation of robust biofilms that are highly adhesive and persistent. Moreover, the biofilm formation in *P. mirabilis* is highly associated with increased rates of antimicrobial resistance and increased expression of toxins[155]. The combination of the aforementioned factors makes *P. mirabilis* a problematic pathogen in DFIs especially chronic ulcers.

Klebsiella pneumonia

Klebsiella pneumonia (*K. pneumonia*) is a Gram-negative bacterium that is commonly isolated from chronic wound infections especially in immunocompromised individuals[158,159]. *K. pneumonia* is known for its high adhesiveness as a result of its thick polysaccharide capsule that is enriched with type 1 and type 3 pili. The polysaccharide capsule in *K. pneumoniae* consists of two fibrous layers: An inner thick densely packed fibrous layer and an outer layer in which the fibers are loosely packed and become finer outwards, forming a fluffy network on the capsule surface[160,161]. This structure plays a leading role in the remarkable adhesiveness of the bacterium onto mucus membranes and inanimate surfaces followed by fast accumulation of bacteria as a result of entangled fibrous polysaccharide capsules of adjacent bacterial cells and subsequently rapid biofilm formation[161,162]. The thickness of the fibrous capsule of *K. pneumonia* is known to be one of the thickest protective bacterial coats, which imparts extra protection against host immune responses such as phagocytosis and serum complement deposition. In

addition, its thick compact nature reduces the penetration of antibiotics and bacteriophages[163,164]. The overall result of the aforementioned factors is the formation of a highly adhesive biofilm that is resistant to immune defenses and antibiotic treatments and makes *K. pneumonia* challenging to eradicate in healthcare facilities, contributing to the high incidence of nosocomial infection associated with this pathogen especially in immunocompromised individuals and diabetic patients[165,166]. It is noteworthy that both *K. pneumonia* and *P. mirabilis* are linked to an increased risk of ascending urinary tract infections in diabetic foot patients as a result of self-infection[167,168].

Enterococcus spp.

Enterococci are facultative anaerobic Gram-positive cocci; there are two species considered the most common commensal organisms in the intestines of humans: *Enterococcus faecalis* and *Enterococcus faecium* [169,170]. *Enterococci* are opportunistic pathogens, commonly responsible for surgical wound infections, urinary tract infections, endocarditis, and intra-abdominal and pelvic infections among many others [171,172]. *Enterococci* are well adapted for withstanding harsh environmental conditions. This enables them to survive routine disinfection methods resulting in high persistence of these bacteria on inanimate surfaces in healthcare settings making them common causative agents of nosocomial infections[172]. It is widely documented that *Enterococci* are among the most prevalent bacterial infections in DFIs[96,117,121,122,124,125,173,174]. Interestingly, *Enterococci* are not considered true pathogens; their abundance in the gut flora provides them the opportunity to interact with other bacteria increasing the possibility of acquiring virulence genes and antimicrobial resistance genes[171, 172]. Lately, there has been an alarming increase in antimicrobial resistance patterns of *Enterococci*, especially associated with hospital-acquired infections affecting immunocompromised patients including DFIs[174]. Unfortunately, many studies reported an increase in the mortality rates related to the emergence of vancomycin-resistant *Enterococci* that are usually linked to hospital-acquired infections [170,171,173]. The current antibiotic choice regimen for control of stubborn multidrug resistant enterococcal DFIs includes antibiotic combinations of β-lactams, aminoglycosides, and fluoroquinolones[171, 174].

MANAGEMENT OF DFIS

Conventional antibiotic therapy guidelines for DFIs

As explained previously, antibiotic treatment should only commence after the confirmation of the presence of an infected wound. However, broad-spectrum antibiotics are typically used during routine care of progressive diabetic foot wounds as an empiric treatment until microbiology culture results are available. Then the treatment should be switched to targeted antimicrobial therapy[175]. Ideally, narrow spectrum antibiotic treatment is preferred in order to avoid antibacterial resistance. Additionally, the treatment should be used for the shortest duration possible in cases of mild and medium diabetic wound infections: For 2-4 wk for progressive wounds and up to 6 wk in cases of osteomyelitis. If the treatment is not effective then the case should be re-evaluated regarding the antibiotic choice[176,177].

The Infectious Diseases Society of America provides a detailed description of antibiotic choices regarding DFIs. However, the report highlights the absence of a single recommended antimicrobial regimen. Instead an appropriate regimen should be designed based on the results of antibiotic susceptibility testing, severity of the infection, possible side effects, price, interactions with other drugs, and other patient related factors. The report recommends including suitable coverage of Gram-positive cocci (mainly *S. aureus* and *Streptococcus* spp.) in empiric treatment protocols. For mild DFIs, the choices include: clindamycin, levofloxacin, and β-lactamase inhibitor combinations. For moderate to severe infections the antibiotic options are extended to include ertapenem, tigecycline, piperacillin-tazobactam combination, and imipenem-cilastatinb combination with the latter showing especially broad spectrum activity. An anti-MRSA agent should be included in the regimen choice in cases of severe infections or previously confirmed MRSA infection. The suggested anti-MRSA choices include: Vancomycin, linezolid, and daptomycin. However, these options are considered narrow spectrum activity, and they should be combined with other agents such as a fluoroquinolone, carpabenem, aztreonam, or piperacillin-tazobactam to increase the activity spectrum especially in severe progressive infections[50,176].

Novel antibiotic options against multidrug resistant DFIs

The fierce increase in antibiotic resistance rates continues to be a growing worldwide crisis, which results in gradual erosion of the list of treatment options available for eradication of multidrug resistant infections, especially DFIs. For example, vancomycin, which is one of the last resort antibiotics that should be conserved for treatment of MRSA, has shown an alarming increase in resistance rates in the last decade[178,179]. Linezolid is considered an effective vancomycin alternative acting against both vancomycin-resistant *S. aureus* and MRSA. Linezolid showed good tissue and bone penetration and sufficient *in vivo* anti-MRSA activity in DFIs, even in cases of blood flow impairment[180,181]. However, linezolid suffers from serious side effects and high toxicity in cases of prolonged treatments. In addition it is not acknowledged by the United States Food and Drug Administration (FDA) for treatment of

osteomyelitis[50,182]. Daptomycin, on the other hand, is approved for intravenous treatment for MRSA in DFIs[106,183]. Additionally, it has a lower side effect profile and promising activity against both MRSA and vancomycin-resistant *S. aureus* that is accompanied by low rates of bacterial resistance development[184,185].

Streptogramins combination of quinopristin and dalfopristin represent another promising alternative treatment of MRSA, which inhibits both the early and the late protein synthesis stages showing significant activity against nosocomial MRSA isolates[186,187]. Tigecycline is a tetracycline derivative that has potent *in vitro* activity against MRSA[186]. However, a Phase III randomized, double-blinded clinical trial showed that tigecycline is significantly less effective and associated with more adverse effects than ertapenem in achieving clinical resolution of DFIs even in presence of osteomyelitis[188]. Ceftobiprole is a fifth generation cephalosporin that is approved for intravenous administration. Ceftobiprole was compared to vancomycin in a multicenter, multinational, double□blind, randomized trial concerning DFIs caused by Gram□positive bacteria. The rates for complete eradication of MRSA in infected patients using ceftobiprole and vancomycin as antimicrobial treatment were 92% and 90%, respectively. In DFI patients, the clinical recovery rate with ceftobiprole monotherapy was 86%, which is as effective as the combination of vancomycin plus ceftazidime[189].

Ceftaroline is another novel cephalosporine that showed significant activity against MRSA. In two randomized, observer□blinded studies to evaluate the efficacy of ceftaroline *vs* standard therapy with vancomycin in combination with aztreonam in adults, the clinical cure rates were comparable (about 86% in both treatments). Importantly, the adverse effects were similar in different treatment groups with a safety similar to that of the cephalosporins[190]. That being said, it is important to bear in mind that any novel antimicrobial treatment, no matter how effective it is against multidrug resistant pathogens, will eventually join the list of ineffective treatments as a result of the continuous evolution of bacterial resistance patterns, which is faster than our ability to develop and approve new alternative treatments.

Topical treatments

Topical antimicrobial treatments of medium to severe DFI wounds are generally considered ineffective [191,192]. Antiseptics are generally applied during surgical debridement procedures and wound dressing changes. This is important to diminish further wound contamination that usually thrives on polymicrobial infections[193]. However, it should be noted that most antiseptics that affect the wound tissues subsequently leave a negative impact on the wound healing process. Furthermore, improper and excessive application of antiseptics can encourage antimicrobial resistance within the wound microenvironment, especially those containing polymicrobial biofilms, thus giving rise to delayed resolution of the infection and increased risk of complications[194]. Based on these considerations, international guidelines do not suggest antiseptics as in the management of DFI wounds[193]. However, several studies documented the *in vitro* effectiveness of iodine-based preparations and dressings containing polyhexamethylene biguanide or silver in controlling DFI wounds[195].

It is reported that biofilm formation within DFIs is likely to increase the incidence of antimicrobial resistance 100 to 1000 times[196], which mandates employment of efficient drug delivery systems to ensure better penetration of the biofilm matrix and higher recovery rates. Some drug delivery suggestions include calcium sulfate beads and antimicrobials immobilized on collagen sponges[196]. Some studies reported a new generation of anti-biofilm hydro-fiber dressings containing carboxymethylcellulose silver, which showed efficient disruption and removal the bacterial biofilms[197].

Another promising dressing was suggested by Yang *et al*[198]. It is a surfactant-based gel dressing that showed promising recovery rates when applied *in vivo* on wounds infected with *P. aeruginosa*. The results showed significant reduction in bacterial growth and disruption in the formed biofilms[198]. Another surfactant-based dressing containing Pluronic F127 in combination with melatonin and chitosan was used to diminish the bacterial growth and biofilm formation in *S. aureus* wound infection [199]. On a similar basis, other studies reported promising *in vitro* antibacterial, anti-biofilm, and healing results upon using wound dressings coated with Chitlac-silver nanoparticles combined with alginate and hyaluronic acid[200].

Other studies went as far as using dressings loaded with mesenchymal stem cells that also showed improved wound healing rates especially in chronic ulcers[201]. The combination of wound dressings with natural products have also been reported in some studies that showed the use of honey[202,203], cranberry extracts[204], tannic acid[205], tea-tree oil[206], and cinnamon oils[207] were linked to improved resolution and healing of DFIs.

Interventional approaches

Surgical debridement is classically used to remove necrotized and infected tissues from DFI wounds. This surgical intervention is routinely used in combination with antibiotics, to control the spread of infection allowing early closure of the wound[208]. The proper removal of infected tissues and bacterial biofilms optimizes the healing and regeneration of the wound tissues, which in turn improves blood flow and improves the effectiveness of the treatment[206]. In association with surgical debridement, negative pressure therapy is commonly employed to promote wound healing in DFIs[209]. Negative pressure is generated using a vacuum source connected to the wound, resulting in suction of cellular

debris, diffuse toxins, and infected extracellular fluids that eventually reflects a positive impact on the resolution of the infection as well as wound healing progress[210].

Photodynamic therapy is a novel technology that is mainly used in oncology. The therapy depends on the use of a photosensitive agent that is activated by illumination to produce lethal oxygen species at the infection site. In a clinical trial, this method was employed for patients suffering from DFIs. The results showed that all the non-treated cases suffered from deterioration of the wound and eventually underwent amputation procedures in comparison to the treated group that showed only 1 case of amputation out of 18 patients who received the photodynamic therapy[211].

Hyperbaric oxygen therapy is another oxygenation-based approach in which pure oxygen is inhaled in a special compression chamber and increases oxygen supply all over the body, including the wound tissues. However, this therapy did not show beneficial results regarding short-term healing of DFI wounds[212].

Novel approaches for treatment of DFIs

The risk of amputation remains significantly high in progressive severe DFIs; such procedures are considered extreme treatment options that usually result in a drastic negative impact on the patient's psychology and productivity in real life. There are numerous new approaches that address this problem by minimizing the need for amputations in severe DFIs. Some of these approaches are discussed in the following points.

Stem cell therapy: One method describes the use of stem cell technology to regenerate the vascular tissues in an ischemic limb, hence increasing blood supply and healing rates in severe DFIs and minimizing the risk of amputations. Additionally, stem cells can be directed towards the release of cytokines, which enhance immunity, cell recruitment, and regeneration of neurons. Similarly, progenitor stem cells can be employed since they have the potential to differentiate into various cell types such as endothelial cells, keratinocytes, pericytes, and myofibroblasts all of which play an effective role in DFI wound healing[213,214]. Stem cell-based therapy has been approved by the FDA as an effective interventional treatment strategy to treat DFI macerated wounds[213]. Secretome stem cells are derived from undifferentiated human mesenchymal endothelial stem cells; they have been successfully deployed for the treatment of the DFIs. It was shown that secretomes enhanced *in vivo* wound healing and increased the proliferation of endothelial cells *via* promotion of the production of a cocktail of vascular endothelial and fibroblast growth factors in addition to angiopoietins[215].

Growth factors: Other approaches are based on the fact that chronic wounds are associated with decreased levels of epidermal growth factor. Hence the application of hormonal growth factors will promote the proliferation and differentiation of fibroblasts, gliocytes, and neo-epidermal cells leading to improved healing rates[213,214]. Other growth factors that modulate signal transduction and replication of epidermal cells were also reported to improve wound healing in DFIs[213,216]. Similar results were obtained upon using granulocyte colony-stimulating factors and human platelet-derived growth factors, which are frequently used for the treatment of DFI wounds and neuropathic ulcers[213].

Skin substitute matrices: One example involves the use of keratinocytes and fibroblasts that are immobilized onto an extracellular matrix that functions as scaffold supports for the wound healing process[217]. Another example is shown by the use of neonatal foreskin equivalent to allogeneic cultured skin apligraf/graftskin. It was shown that this supportive tissue significantly improved the healing of chronic wound ulcers[218]. Dermagraft is an isolated neonatal human dermal fibroblast. Its application significantly improved the healing rates up to 30% in DFI wounds[219]. Furthermore, the allogeneic membranes obtained from human placenta have been employed successfully in the treatment of DFI wounds; such membranes provide growth factors, cytokines, and structural collagen support, which improved the repair of deteriorated tissues[220]. Furthermore, allografts from human skin such as GraftJacket were also reported as successful scaffolds for support of vascular and cellular growth in severe wounds[213].

Phage therapy: Phage therapy is an old method that is starting to gain renewed worldwide attention. The method is based on the use of bacteriophages, which are viruses that infect bacteria. Bacteriophages are considered the natural predator of bacteria that are abundant in nature[221,222]. Phage therapy usually uses a cocktail of bacteriophages to increase the host spectrum range[223]. In one *in vitro* study, a phage cocktail was designed to target *S. aureus, P. aeruginosa,* and *Acinetobacter baumannii* isolated from DFIs. The results showed significant antimicrobial and anti-biofilm activity of the tested bacteriophages [224]. These results were supported by case reports that encourage phage therapy for DFIs[225,226]. Examples of *in vitro* tested bacteriophages against the most prevalent bacterial species in DFIs are listed in Table 1.

The use of bacteriophages for treatment of pathogenic bacterial infections offers many advantages: (1) High specificity of action because bacteriophages are highly specific in selection of their host, which is usually limited to one species or even one specific strain within a species; (2) Can be used against multidrug resistant bacteria because bacteriophages use a pathway that is different from all antimicrobial treatments. Therefore, most resistance mechanisms will not affect the phage pathway; (3) Phages

Table 1 Examples of anti-biofilm and anti-virulence agents against the most prevalent diabetic foot infections bacterial pathogens

Agent	Target microbe	Ref.
Bacteriophages		
vB_SauM_ME18 vB_SauM_ME126	*S. aureus*	[246]
Bacteriophage K	*S. aureus*	[247]
pSp-J and pSp-S	*Staphylococcus* spp.	[248]
Staphylococcus bacteriophage K	*S. epidermidis*	[249]
Bacteriophage cocktail	*P. aeruginosa*	[250]
Pseudomonas Phage	*P. aeruginosa*	[251]
vB_EcoS-Golestan	*E. coli*	[252]
Lytic bacteriophage cocktail	*P. mirabilis, E. coli*	[253]
Bacteriophage cocktail	*P. mirabilis*	[254]
vB_PmiS-TH	*P. mirabilis*	[255]
PhiS1	*P. aeruginosa*	[256]
PhiE2005-A	*P. aeruginosa*	[257]
Lytic bacteriophage	*K. pneumonia*	[258]
Anti-biofilm and Anti-virulence agents		
Sitagliptin (anti-diabetic)	*P. aeruginosa*	[46,235,259]
	S. aureus	[46]
Linagliptin	*P. aeruginosa*	[238]
Metformin (anti-diabetic)	*P. aeruginosa*	[45,236]
Diclofenac (analgesic)	*P. mirabilis*	[239]
Metronidazole (antibacterial)	*P. mirabilis*	[260]
Fluoxetine (antipsychotics)	*P. mirabilis*	[261,262]
Thioridazine (antipsychotics)		
Penfluridol (antipsychotics)	*E. faecalis*	[263]
Terazosin (adrenoreceptor blockers)	*P. aeruginosa*	[231,264]
Prazosin (adrenoreceptor blockers)	*P. aeruginosa, P. mirabilis*	[48,265,266]
Metoprolol (adrenoreceptor blockers)	*P. aeruginosa, S. enterica*	[233,267]
Atenolol (adrenoreceptor blockers)	*P. aeruginosa, P. mirabilis*	
Allopurinol (anti-gout)	*P. aeruginosa*	[47]
Azithromycin (antibiotic)	*P. aeruginosa*	[268]
Ciprofloxacin (antibiotic)	*S. enterica*	[269]
Resveratrol (anticancer)	*S. aureus*	[270]
	E. coli	[271]
Ribavirin (antiviral)	*C. albicans*	[272]
Theophylline (bronchodilator)	*C. albicans*	[273]
Stolon (fenugreek)	*P. aeruginosa*	[232]
Garlic extract	*P. aeruginosa*	[274]
Allicin (garlic)	*P. mirabilis*	[275]
Carvacrol (oregano)	*P. aeruginosa*	[276]
Emodin (*Polygonum cuspidatum*)	*S. aureus*	[277]
	C. albicans	[278]

Curcumin (curcuma)	*Acinetobacter baumannii*	[279]
	C. albicans, P. mirabilis	
Tannic acid	*E. coli*	[280]
Sodium citrate	*P. aeruginosa*	[281]
Isolimonic acid (citrus fruits)	*E. coli*	[282]
Zingerone (ginger)	*P. aeruginosa*	[283]

S. aureus: *Staphylococcus aureus*; *S. epidermidis*; *Staphylococcus epidermidis*; *P. aeruginosa*; *Pseudomonas aeruginosa*; *E. coli*: *Escherichia coli*; *P. mirabilis*: *Proteus mirabilis*; *K. pneumonia*: *Klebsiella pneumonia*; *E. faecalis*: *Enterococcus faecalis*; *S. enterica*: *Salmonella enterica*; *C. albicans*: *Candida albicans*.

will only attack the target bacterial host leaving no effect on eukaryotic cells, which means localized activity at the infected tissues with minimal side effects; (4) Self-amplification of phages means that minimal doses will replicate exponentially at the infection site in relation to the wound infection burden; (5) High ability to penetrate deep tissues and bacterial biofilms, which further results in complete eradication of the infection; and (6) Minimal effect on the normal host flora[227]. On the other hand, there are limitations, mainly the lack of approval from the FDA and the need to formulate a phage cocktail that is based on accurate identification of polymicrobial infection members[227]. Moreover, it was observed that biofilm formation was induced by exposure to some phages[228,229].

Anti-biofilm and anti-virulence agents: Bacterial biofilms and bacterial virulence play major roles in the establishment and spread of DFIs. Anti-biofilm and anti-virulence agents are promising adjuvants to be used in combination with conventional antibiotic treatment of DFI wounds[206]. Bacteria employ several interplaying systems to control the expression of their virulence factors, most importantly the QS system. QS is used in both Gram-positive and Gram-negative bacteria to communicate between each other in an inducer-receptor manner[37,40,46]. Several approaches have been suggested to diminish the bacterial biofilm formation and virulence factor production based on targeting the QS systems[47,69, 71]. QS inhibitors are known to reflect a significant reduction in bacterial virulence as well as reduced resistance development[230-234].

There are several chemical structures that have been screened for their anti-QS, anti-biofilm, and anti-virulence activities, with maximum attention given to the screening of already used and approved medications with the aim of using them for other applications than their originally intended use (Table 1). Some of the screened drug groups included several anti-diabetic agents. Fortunately, some anti-diabetics showed promising anti-QS, anti-virulence, and anti-biofilm activities. One promising example is the group of gliptins, which are dipeptidase inhibitors that are widely used as hypoglycemic agents. A detailed virtual study was performed to assess the anti-QS activity of some gliptins, mainly sitagliptin and linagliptin[46,235-238]. The results showed a significant ability to diminish biofilm formation is *S. aureus* and *P. aeruginosa* in addition to significant reduction in the expression of virulence factors such as protease, hemolysins, and other toxins[45,46,238]. There is a growing list of drug groups that are screened for their antibacterial and anti-QS activities, including analgesics and anti-inflammatory agents that are commonly used for symptomatic treatment of DFIs. Diclofenac is a commonly used anti-inflammatory agent that showed promising *in vitro* results regarding biofilm inhibition and downregulation of virulence factors in *P. mirabilis* isolates collected from deep DFIs[239]. There are many other drug groups and natural products that were screened for their anti-QS, anti-biofilm, and anti-virulence activities. Some of these agents are presented in Table 1.

There are other approaches that aim at inhibition of bacterial biofilm formation, for example chelation of essential metals, ethylene diamine tetra-acetic, and citrate[240]. Another approach is the use of enzymes for dispersion of bacterial biofilm, *e.g.,* α-amylase[241], proteinase K, trypsin[206], deoxyribonuclease I, hydrolases, and DNase[241-243]. In addition, some synthetic chemical agents such as 2-aminoimidazole showed powerful anti-biofilm activity against *S. aureus*[244].

In another study published by Barki *et al*[245], wireless electroceutical dressings were used successfully for the eradication of *P. aeruginosa* and *Acinetobacter baumannii* biofilms *in vivo*. It was shown that the dressing disrupted the formed biofilms and accelerated wound healing. Furthermore, this treatment was found to downregulate the QS-encoding genes and restore the skin barrier function by silencing the proteins required for skin barrier function (E-cadherin)[245].

CONCLUSION

Diabetes and its complications represent a growing public concern worldwide. DFIs are considered one of the most commonly encountered problems at healthcare facilities. The management of DFIs are usually problematic due to many factors, including the reduced immunity in diabetic patients, the

delayed wound healing, and the high incidence of a multidrug resistant polymicrobial infection. The delay or failure of treatment of DFIs will increase the risk of serious life-threatening complications such as amputations and systemic infections. There has been a global increase in the levels of bacterial resistance to antibiotics that reached a catastrophic level, especially with more and more antibiotics being added to the list of ineffective treatments. This has caused increased rates of mortalities caused by multidrug resistant infections. The proper selection of the antibiotic treatment course for DFI is crucial to avoid microbial resistance. Additionally, it is important to combine antimicrobial treatment with supportive therapy such as anti-biofilm agents, drug delivery systems, and rejuvenating dressings to ensure maximum outcomes of the treatment. In addition, the use of QS inhibitors will decrease the severity of the infection by downregulation of bacterial virulence factors, biofilm formation, and reduction of the incidence of antimicrobial resistance.

FOOTNOTES

ORCID number: Azza A H Rajab 0000-0001-9018-6002; Wael A H Hegazy 0000-0001-5683-4803.

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Food contaminants and potential risk of diabetes development: A narrative review

Maja Milanović, Nataša Milošević, Nataša Milić, Milica Medić Stojanoska, Edward Petri, Jelena Marković Filipović

Maja Milanović, Nataša Milošević, Nataša Milić, Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad 21000, Serbia

Milica Medić Stojanoska, Faculty of Medicine, Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Center of Vojvodina, University of Novi Sad, Novi Sad 21000, Serbia

Edward Petri, Jelena Marković Filipović, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad 21000, Serbia

Corresponding author: Jelena Marković Filipović, PhD, Associate Professor, University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, Novi Sad 21000, Serbia. jelena.markovic@dbe.uns.ac.rs

Abstract

The number of people diagnosed with diabetes continues to increase, especially among younger populations. Apart from genetic predisposition and lifestyle, there is increasing scientific and public concern that environmental agents may also contribute to diabetes. Food contamination by chemical substances that originate from packaging materials, or are the result of chemical reactions during food processing, is generally recognized as a worldwide problem with potential health hazards. Phthalates, bisphenol A (BPA) and acrylamide (AA) have been the focus of attention in recent years, due to the numerous adverse health effects associated with their exposure. This paper summarizes the available data about the association between phthalates, BPA and AA exposure and diabetes. Although their mechanism of action has not been fully clarified, *in vitro*, *in vivo* and epidemiological studies have made significant progress toward identifying the potential roles of phthalates, BPA and AA in diabetes development and progression. These chemicals interfere with multiple signaling pathways involved in glucose and lipid homeostasis and can aggravate the symptoms of diabetes. Especially concerning are the effects of exposure during early stages and the gestational period. Well-designed prospective studies are needed in order to better establish prevention strategies against the harmful effects of these food contaminants.

Key Words: Acrylamide; Bisphenol A; Phthalates; Endocrine disrupting chemicals; β-cell; Diabetes

Core Tip: One of the most important steps in the prevention and control of diabetes and related disorders is the identification of potential risk factors. Phthalates, bisphenol A (BPA) and acrylamide (AA) are chemicals that are ubiquitously present in the environment and have the ability to act as contributing factors with adverse health effects. Human exposure to phthalates, BPA and AA mainly occurs through ingestion. This paper summarizes the available data about the association between phthalates, BPA and AA exposure and diabetes in order to examine the potential role of these contaminants in the development and progression of this complex disorder.

INTRODUCTION

One of our basic human rights is "the right of everyone to have access to safe and nutritious food"[1]. According to the World Health Organization, more than 100 billion dollars is spent each year on medical expenses related to the consummation of unsafe food around the world[2]. Food contamination by chemical substances is generally recognized as an emerging worldwide challenge, with potential health hazards[3,4]. Moreover, diet has been identified as a main source of chemical intake[5]. Chemicals may enter the food chain *via* several pathways during cultivation, production, handling and processing, packaging, transportation and storage[4]. Numerous studies have confirmed the presence of a wide range of chemicals in drinking water, fruits, vegetables, cereals, meat and poultry, seafood, canned food, dairy products, baked goods, fast foods *etc.*[6-10]. For instance, humans are exposed daily to multiple chemicals, including environmental and processing contaminants, that may pose a threat to health even at very low concentrations[11]. The continuous ingestion of chemicals that migrate from food packaging, especially plastic packaging materials, or that are the result of chemical reactions during food processing, can lead to adverse health effects such as the development of diabetes.

Among the chemicals that originate from plastic packaging materials, endocrine disrupting chemicals (EDCs) have attracted public attention due to their possible harmful health effects[12]. The Endocrine Society classified EDCs as "a serious public health risk" and since then, data demonstrating their negative effects on human health has been constantly increasing. To date, more than 1400 chemicals have been identified as potential EDCs[13]. EDCs are xenobiotics that interfere with normal endocrine function, which consequently lead to adverse health outcomes[14-17]. Phthalic acid esters (PAEs) and bisphenol A (BPA) are well-known EDCs that are found practically "everywhere" in human societies, and have been the focus of scientific and public attention in recent years.

Among the chemical substances that are inadvertently generated during food preparation, acrylamide (AA) has raised public health concerns since it was first detected in 2002. Over the past twenty years, AA has been recognized as a "potential human carcinogen", an emerging food contaminant and potential EDC[18,19]. Based on the above, exposure to PAEs, BPA and AA has been associated with a range of adverse health outcomes. Considering that ingestion is the main route of exposure, the objective of this paper is to review the current data concerning the links between PAEs, BPA and AA exposure and diabetes, in order to better understand the potential roles of these compounds in the development and progression of this complex disorder (Figure 1).

DIABETES

A century after the discovery of insulin, diabetes has been transformed from a fatal disorder into a chronic condition[20]. Today, the number of people diagnosed with diabetes continues to increase exponentially and it has been predicted that by 2045 more than 780 million people will have diabetes; with type 2 diabetes (T2D) representing approximately 90% of the total number of cases. It is believed that as many as half of the total number of cases remains undiagnosed, especially in low-income and middle-income countries[21] and that diabetes and its complications have resulted in more than 6.5 million lost lives over the last year alone[21]. In the United States, it is estimated that the non-health costs of diabetes per person per year surpass the costs of heart diseases[22]. Therefore, recognition of potential risk factors is one of the most important steps in the establishment of efficient strategies for the prevention and control of diabetes and related diseases that will consequently reduce the burden on the
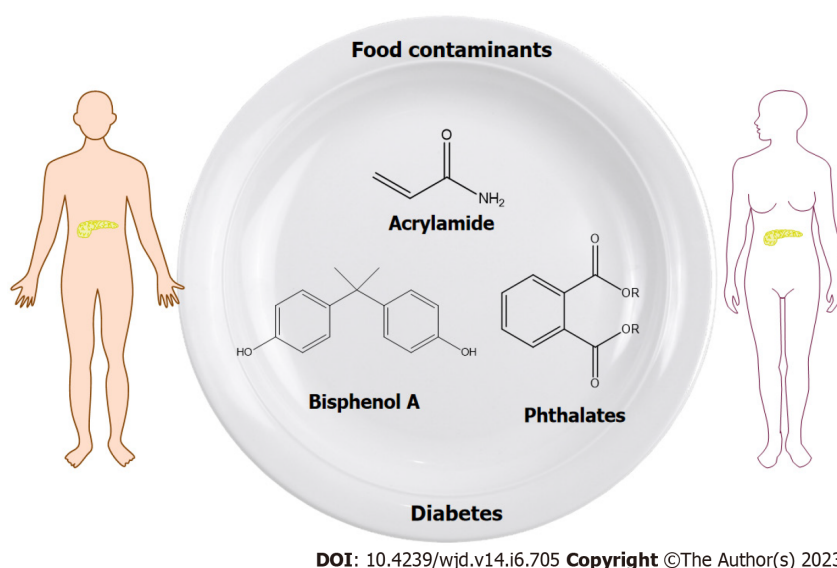
Figure 1 Selected food contaminants are represented with their chemical structures as potential risk factors for diabetes development.

healthcare system and society.

Diabetes is a chronic disease, associated with a range of metabolic abnormalities. The clinical manifestations of diabetes includes increased serum glucose levels, which are a consequence of insulin deficiency and/or insulin resistance[23]. Type 1 diabetes (T1D) refers to a chronic autoimmune disease characterized by the loss of pancreatic β-cells, which leads to a total lack of insulin secretion and results in elevated blood glucose levels[24,25]. Although the development of T1D is associated with a genetic predisposition, environmental agents (single compounds or mixtures of compounds) can activate autoimmune mechanisms involved in the development of this multi-factorial disorder, through mechanisms that are not completely understood[26]. Insulin resistance is identified as a "key player" in the development and progression of T2D[27]. T2D is known as "adult-onset diabetes", and develops as a result of increased insulin resistance to a level where overproduction of insulin can no longer cope with insulin insensitivity, leading to β-cell dysfunction[28]. In addition, several other non-communicable disorders are associated with insulin resistance, such as obesity, metabolic syndrome, non-alcoholic fatty liver disease, polycystic ovary syndrome, cardiovascular disease and cancers[20]. However, there is a growing amount of data that also supports a role for food contaminants, such as PAEs, BPA and AA in the onset of diabetes and the development of related conditions.

PAES

Overview

PAEs are one of the most commonly used plasticizers and additives in a wide-range of products, such as food packaging, detergents, cosmetics, toys, medical tubing, blood-storage containers, and home furnishings. Due to the ability of phthalates to improve the mechanical properties of polymers (*e.g.*, polyethylene, polyethylene terephthalate, polyvinyl acetate and polyvinyl chloride), it is predicted that approximately 500 million tons of PAEs will be produced worldwide by 2050[12,29-31]. Some of the most frequently used PAEs are dimethyl phthalate, diethyl phthalate (DEP), di-n-butyl phthalate (DBP), diisobutyl phthalate (DiBP), di-n-hexyl phthalate, bis (2-ethylhexyl) phthalate (DEHP), diisononyl phthalate, di-n-octyl phthalate and benzylbutyl phthalate[30,32]. Because of their large production volume and widespread applications, these PAEs are omnipresent contaminants[33]. Since PAEs are weakly bound to plastic polymers, they are easily released into the surrounding environment (*i.e.*, in food, water, air, soil) during production, storage, use and disposal of plastic-based products[34]. Because of this, PAEs can be frequently detected in different biological and environmental matrices such as urine, blood, air, soil, sediment, food, surface water and even drinking water[35-40]. The bioaccumulation and biodegradation potential of PAEs is dependent on their physico-chemical properties, which consequently determine their behavior and fate in the environment and their toxicity[41]. Phthalates are associated with negative effects on human health, including obesity, dyslipidaemia, T2D, impaired thyroid function, breast and uterine cancer, endometriosis and low birthweight[42-48]. Upon entering the food chain, the main route of humane exposure to phthalates is by ingestion. In the European Union, it is forbidden to use phthalate-containing materials for infant food and goods which contain high amounts of fats, such as dairy products. Moreover, since January 2022, plastic packaging for fruits and vegetables has been banned in France[49]. DEHP has been estimated as "safe" under 4.8 mg/kg body

weight per day (no-observed-adverse-effect level) while the tolerable daily intake (TDI) is 0.05 mg/kg body weight per day[50]. However, data concerning PAE contamination levels in different components of the human diet, especially with respect to vulnerable populations, remains scarce and limited. Hence, PAE-related health risks cannot be neglected even at the "safe dose" exposure levels defined by regulators. Considering that PAEs show additive effects, particular attention must also be given to the potential synergistic effects of mixtures of EDCs[51].

PAEs and diabetes

Research status: As EDCs, PAEs have the ability to modulate the activity of multiple nuclear receptors, such as estrogen receptors (ERα and ERβ), androgen receptor (AR), peroxisome proliferator-activated receptors (PPARα and PPARγ), thyroid hormone receptors (TRα and TRβ) and the pregnane X receptor [15,52,53]. In order to understand the connection between PAEs and diabetes, "the dose makes the poison" approach cannot be applied[54]. Although phthalate exposure or mixed exposure with BPA had no influence on T1D development in non-obese mice, a mixture of PAEs and BPA decreased the release of tumor necrosis factor α (TNFα), interleukins (IL-4, IL-6, IL-10) and interferon γ in splenocytes and pancreatic lymphocytes and caused impairment of the immune system[55]. A significant association between PAE exposure and diabetes was probably not observed, due to the use of PAEs in high doses. PAEs as EDCs show non-monotonic effects[56]. Estrogenic compounds in high doses trigger insulin secretion in β-cells, and thus postponed the development of diabetes in non-obese mice[57]. In contrast, administration of DEHP at low levels caused the onset of diabetes symptoms (decrease in serum insulin levels and liver glycogen and an increase in blood glucose levels) followed by thyroid and adreno-cortical dysfunction in rats[58]. After oral intake, PAEs undergo two metabolic steps. Short-branched phthalates are hydrolysed into monoester metabolites (mPAEs) and extracted *via* urine; while after several biotransformation steps in the first phase, long-branched phthalates are conjugated in phase II and eliminated through urine and feces[59]. Therefore, mPAEs should be also considered in order to understand the association between exposure to PAEs and diabetes. Based on *in vitro* and *in vivo* studies, mPAEs are more potent at a molecular level compared to their parent diester compounds[60-62]. PAEs and mPAEs have affinity for PPARs receptors, which are involved in complex mechanisms of regulation of glucose homeostasis, insulin sensitivity, differentiation of adipocyte and adipogenesis[63]. However, when the effects of BPA and three phthalate metabolites [monoisobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), and mono-(2-ethylhexyl) phthalate (MEHP)] were investigated in pancreatic β-cells at concentrations of 5-500 μM, BPA treatment resulted in a more significant decrease in cellular viability after 72 h of exposure. Although increased insulin secretion was observed for BPA, MEHP, and MnBP after 2 h of simultaneous exposure to chemicals and glucose, no effects on glucose promoted insulin secretion were obtained after exposure for 24-72 h[64]. In contrast, when rats were treated orally with DEHP throughout gestation and lactation, abnormalities in β-cell ultrastructure, together with a decrease in β-cell mass and insulin content in the pancreas were found. Also, in DEHP treated offspring, alterations in pancreas specific gene expression were observed and impairment in β-cell development and function were reported[65]. Particularly, a decrease in the levels of pancreatic and duodenal homeobox-1 (Pdx-1) were observed in DEHP exposed rats of both sexes, as well as an increase in genes involved in endoplasmic reticulum stress, when compared to controls[65]. Considering the fact that Pdx-1 is involved in regulation of insulin gene expression, glucokinase, glucose transporter 2 (GLUT2), islet amyloid polypeptide and somatostatin, Pdx-1 plays crucial roles in the development of β-cells features and functions[66]. Therefore, this decrease in Pdx-1 activity is probably one of the principal mechanisms of DEHP-induced dysregulation of pancreatic β-cells[67]. DEHP exposed offspring had increased blood glucose levels and decreased pancreatic insulin levels and displayed changes in glucose tolerance and glucose stimulated insulin secretion. Despite this observed β-cell dysfunction and wide range of glucometabolic changes, DEHP exposure during the gestational period also induced epigenetic changes and led to inhibition of β-cell development[68]. Particularly, in both sexes a significant decrease in the levels of glucokinase mRNA was observed, which correlated with applied DEHP dose. Moreover, endoplasmic reticulum stress markers were increased, along with the concentrations of plasma membrane bound GLUT2 protein[68]. In addition, DiBP reduced fetal plasma insulin levels in offspring and decreased PPARα mRNA levels in the liver[69]. Additionally, gender and weight differences related to DEHP and diabetes development were seen in adulthood. Namely, DEHP exposed female offspring had lower birth weights, disturbed glucose tolerance, impaired insulin secretion and high blood glucose levels. DEHP exposed male offspring had increased serum insulin levels and lower birth weights at a significant level[65]. When compared to DBP, DEHP induced pancreatic dysfunction and inhibition of insulin secretion was more pronounced in the offspring of rats after *in utero* and lactational exposure to phthalates[70]. Relative to the effects of DEHP exposure in normal mice and male T2D mice in puberty, female T2D mice in puberty were more sensitive to DEHP. Namely, in DEHP exposed female T2D mice during puberty, higher levels of several parameters were detected such as insulin, C-peptide, fasting blood glucose levels, homeostatic model assessment of insulin resistance (HOMA-IR), low density lipoprotein, C-reactive protein and aspartate aminotrans-ferase (AST). Also, DEHP triggered oxidative stress in terms of higher malondialdehyde (MDA) content and lower superoxide dismutase (SOD) and glutathione (GSH) peroxidase activity in the livers of both normal and T2D mice[71]. DEHP promoted increased body weight in normal adolescent mice. Increases

in fasting blood glucose levels and glycated hemoglobin A1c (HbA1c) were more pronounced in adolescent T2D mice in comparison with normal adolescent mice. Additionally, DEHP induced insulin secretion and insulin resistance in normal adolescent mice, inhibited glycogen synthesis in adolescent T2D mice, and caused a decrease in the serum-lecithin cholesterol acyltransferase and hepatic lipase levels. A reduction in insulin levels was found in DEHP-treated adolescent T2D mice[72]. In both DEHP treated groups, a decrease in the expression of insulin receptors (IR-β and IRS-1) and GLUT4 was detected. Hence, DEHP acts as a metabolic toxicant in T2D development through impairment of glucose and lipid metabolism, and disruption of β-cell function and development[72]. Additionally, metabolic toxicity and insulin resistance caused by DEHP were more pronounced in rat liver cells with insulin resistance compared to normal cells[73]. In both cell lines, DEHP promoted cell damage through increased lipid peroxidation, alanine transaminase and AST levels, caspase-3 levels as a marker of cell apoptosis, and downregulated levels of IR-β. DEHP triggered macrophage infiltration in rat adipose tissue and stimulated the production of TNFα and IL-1β, promoting inflammation, while impairing normal lipid metabolism[74].

Potential mechanisms associated with diabetes: Although the mechanism of action of PAEs in diabetes has not been fully clarified, *in vitro* and *in vivo* studies have made significant progress toward identifying an association between PAE exposure and the development of diabetes. Interactions of PAEs with PPARs receptors impaired molecular signaling pathways (*i.e.*, downregulated Pdx-1, activated JNK and caspase-3 expression, inhibited extracellular signal-regulated kinase (ERK)1/2, activated JAK/STAT pathway, and affected neuropeptide Y expression) that have a significant role in the regulation of glucose and lipid homeostasis[65,68,71,74,75]. Therefore, PAEs induce mitochondrial dysfunction, inflammation and increased oxidative stress, while decreasing the levels of IRs and GLUTs. PAEs also promote β-cell dysfunction, apoptosis, impaired insulin sensitivity and glucose cell uptake, and consequently cause glucometabolic and lipid abnormalities (Figure 2). In addition to their role in the onset of diabetes, PAEs act as obesogenic and diabetogenic chemicals that can aggravate the symptoms of diabetes. Especially concerning is the fact that prenatal PAEs exposure is a potential risk factor for developing diabetes, and pre-clinical studies imply that women are most susceptible to the adverse effects of PAEs.

Epidemiologic evidence: The relationship between PAE exposure and potential risk for development of diabetes has mostly been examined by cross-sectional studies that differ in the race, gender and ages of study participants, sampling size, type of matrix, analytical techniques and kind of phthalates and/or metabolites used as analytes[76-82]. Because PAEs undergo quick metabolism and are excreted *via* urine as conjugated monoesters, evaluation of mPAEs concentrations in urine is most appropriate for assessment of possible correlations between PAE exposure and diabetes in humans[83]. Different types of PAEs have similar structures and mechanisms of action, and thus their negative effects may be additive[51]. Hence, the sum of phthalate metabolites should be considered as well during assessment of their negative effects[43]. The first evidence for the diabetogenic potential of PAEs in the human population was reported almost 15 years ago in a study where positive correlations were found between mPAE concentrations, abdominal obesity and insulin resistance in males[84]. Although urinary mPAEs concentrations were not associated with T1D at a significant level, in children with new-onset T1D, higher concentrations of MiBP were detected[76]. A high frequency of DEP and DEHP detection in urine was observed in healthy adults, the obese, and people with newly diagnosed T2D[34]. Higher urinary mPAEs levels, especially monomethylphthalate (MMP), MEP and MiBP, were related to a higher prevalence of T2D in both sexes[78,82]. Particularly, MEP and MMP were associated with insulin resistance, while MiBP was correlated with low insulin secretion[82]. Moreover, the association between mPAE concentrations and T2D was more pronounced in young individuals in comparison to older individuals. Interestingly, a positive correlation between specific urinary mPAEs and HbA1c levels was observed in individuals with a lower body mass index, while MEHP concentrations were positively related to fasting glucose levels in men and in the elderly[77]. Additionally, MEHP levels were associated with glucose serum levels in T2D patients and urinary MEP concentrations were positively correlated with HOMA-IR while in healthy participants, positive correlations were found between urinary MEP levels and triglyceride glucose index and triglyceride glucose-body mass index[43]. Both parameters have been proposed as indicators of T2D development in healthy normoglycemic participants[85]. It was found that higher concentrations of specific mPAEs were associated with increased oxidative stress and inflammation in diabetic patients in terms of MDA and TNFα levels, and decreased adiponectin levels[86]. Based on a non-targeted metabolomic study, differences in the serum levels of biomarkers of galactose, amino acids and pyrimidine metabolism were observed between T2D and control groups and mPAEs levels were mostly significantly associated with metabolic biomarkers serum concentrations[87].

In order to examine prospective evidence concerning the association of phthalates with T2D, cohort studies were performed. It was found that, among middle-aged women, T2D may be associated with phthalate exposure[88]. *In utero*, MEP exposure was associated with poor insulin secretion among pubescent boys, while increased leptin was observed among girls. *In utero*, and during the peripubertal period, DEHP exposure was associated with higher serum insulin-like growth factor-1, insulin
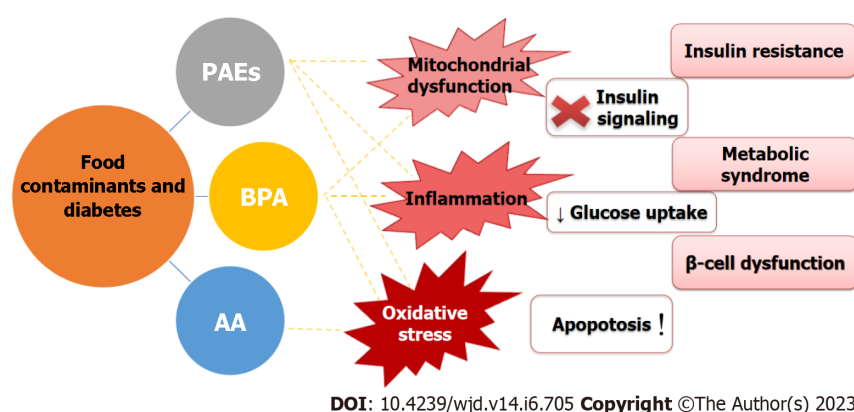
Figure 2 Schematic mechanisms of phthalates, bisphenol A and acrylamide role in diabetes development. PAEs: Phthalic acid esters; BPA: Bisphenol A; AA: Acrylamide.

secretion, and insulin resistance[89]. Moreover, in order to better investigate the link between specific phthalates and their metabolites with diabetes, several meta analyses were recently performed. DEHP exposure is mostly related to insulin resistance[90] and a positive correlation was found between phthalate metabolites and increased HOMA-IR[91],while the presence of MMP, MnBP, MiBP, mono-(3-carboxypropyl) phthalate in urine were positively associated with risk of diabetes[92]. Results obtained from epidemiological studies provide additional evidence about the negative effects of phthalates on glucose and lipid metabolism.

BPA

Overview
BPA is one of the most well-known EDCs because of the numerous adverse health effects associated with its widespread application in different everyday products. BPA is used in the production of polycarbonate plastics and epoxy resins, and can be found in plastic containers and cans for food and beverages, numerous kitchen appliances and utensils, personal care products, toys, paints, electronics, sports equipment, medical devices, dental materials and thermal paper[93,94]. Because of its known reproductive toxicity and endocrine disruption potential, the use of BPA in baby bottles and toys is forbidden in the United States, Canada and the European Union[95]. However, despite continuous debate over more efficient measures to protect especially vulnerable populations from BPA exposure, BPA production and consumption is still increasing. It is expected that BPA commercial sales will exceed 30 billion USD in 2028[96]. Similarly, to PAEs, food can be contaminated with BPA during production, handling, packaging, and transportation[97]. BPA migration from container linings may be increased under high temperature, acid or basic conditions and even due to microwave exposure[95]. Hence, diet is recognized as a main source of BPA exposure, particularly the ingestion of BPA *via* canned foods[98]. Although the European Food Safety Authority has set a reduced TDI for BPA (0.04 ng of BPA per kg body weight per day), the daily intake of BPA through the diet is several times higher (0.17-0.95 μg of BPA per kg body weight per day)[99]. An extensive number of studies has documented the association between BPA exposure and increased oxidative stress, fertility disorders, obesity in children, adolescents and adults, metabolic disturbances and impaired pancreatic β-cell function, as well as cardiovascular diseases and even increased carcinogenicity[100-106].

BPA and diabetes
Research status: BPA is classified as a "weak estrogen" and "obesogen" due to its endocrine disruptive potential, which is mainly a result of the known ability of BPA to bind to nuclear receptors[15,107]. Acute and long-term effects of low BPA concentrations on the development of diabetes have been documented. Enhanced insulin synthesis was observed through the interaction of ERα with ERK2 in pancreatic β-cells[108]. Similar to 17β-estradiol, picomolar doses of BPA trigger Ca^{2+} signaling pathways leading to insulin secretion in pancreatic β-cells. In addition, BPA exposure may cause inhibition of the expression of Pdx-1 in pancreatic mice islets, resulting in a decrease in glucose promoted insulin secretion and ATP production. Moreover, microRNA expression and BPA induced insulin secretion dysfunction in pancreatic islets has also been studied. Particularly, BPA suppressed the expression of miR-338, resulting in down-regulation of Pdx-1[109]. The "inverted U-shaped dose-effect curve" corresponds to the impact of BPA on insulin secretion in β-cells and mitochondrial function[110]. It is

worth noting that more pronounced effects were exhibited by BPA binding to ERβ receptors. BPA as an insulinotropic pollutant affected β-cell function through inhibition of K(ATP) channel activity, which was observed in ERβ+ mice and human β-cells and islets[111,112]. In comparison with phthalate metabolites (MnBP, MiBP, and MEHP), BPA more strongly affected viability and insulin secretion in pancreatic β-cells[64]. However, in the same study, cytokine-induced cell death, a marker of T1D, was not affected. In spite of this, BPA was found to aggravate T1D in mice by disturbing Ca^{2+} signaling; indicating that BPA may cause insulin resistance *via* exacerbation of endoplasmic reticulum stress in pancreatic β-cells[113]. The diabetogenic potential of BPA has also been documented in insulinoma cell lines, where increased insulin secretion was observed together with decreased cell viability at nanomolar BPA levels[114,115]. BPA induced insulin hypersecretion was associated with enhanced β-cell lymphoma 2 family members, caspases and mitochondrial stress, which led to apoptosis[114]. Additionally, apoptosis may be promoted through BPA induced formation of amyloid fibrils. In rat insulinoma cells, BPA at micromolar concentrations induced DNA damage *via* increased levels of the proteins p53 and p-Chk2, as well as increased production of reactive oxygen species and decreased GSH levels[116]. In pancreatic α-cells, which are responsible for glucagon secretion, BPA reduced the fluctuation of low glucose levels induced by Ca^{2+}[117]. To date, there is no published data concerning the impact of BPA on other Langerhans islets cells (δ, γ, ε). Regarding the data about BPA's role in autoimmune related disorders, such as T1D, the effects of low and high doses of BPA on T-cell immunity mechanisms have also been examined. Results show that at low doses, BPA acts as a promotor of diabetes, both through modulation of CD4+ T-cells and production of interferon γ, IL-6 and TNFα[118]. BPA effects were not sex-dependent, based on the experiments performed in non-obese diabetic mice models[119]. However, exposure to BPA during the prenatal stage is particularly dangerous, considering that BPA increased the risk for T1D development and metabolic disturbances in juvenile mice models and adult mice offspring, respectively[119,120]. Additionally, changes in gut microbiota and inflammation were recorded in juvenile mice[119,121]. Prenatal BPA exposure during the lactation period led to an increase in body weight in mice[122]. Even at "safe" levels (below the predicted 'no adverse effect' concentration) prenatal BPA exposure led to a significant increase in body and liver weight, abnormalities in adipocytes in terms of mass, number and volume, as well as elevated serum leptin and insulin levels, together with a decrease in adiponectin and glucose tolerance in adult male offspring[120]. Also, BPA exposure during lactation induced body weight gain in mice[122]. In pregnant BPA exposed mice, insulin resistance, together with elevated levels of insulin, triglycerides, and leptin in plasma, as well as glucose intolerance were observed[123]. Prenatal BPA exposure had detrimental effects on β-cells in mice, in terms of cell growth, mass and proliferation[124]. Therefore, exposure during early stages and the gestational period may cause long-term vulnerability to metabolic diseases and the development of glucose intolerance as a collateral effect or through epigenetic modifications[125,126].

Potential mechanisms associated with diabetes: The mechanisms of action of BPA are complex. Besides impairment of β-cell function, pre-clinical studies suggest that BPA is involved in the production of insulin resistance promoters, such as IL-6 and TNFα and inhibition of adiponektine in adipose tissue. In addition, BPA is associated with increased lipid peroxidation and pro-inflammatory cytokines in hepatocytes, as well as alterations in signaling pathways that generate reactive oxygen species, affect T-cell immunity, leading to decreased insulin sensitivity in skeletal muscles and glucose tolerance in the liver (Figure 2)[127-134].

Epidemiologic evidence: Evidence for the diabetogenic effects of BPA could not be completed without biomonitoring studies. Considering that free BPA has higher affinity for nuclear receptors than glucuronide and sulfate conjugates, the adverse effects of BPA are still evaluated mostly by measuring total BPA levels in urine, as a matrix of choice, and are expressed as creatinine-adjusted mean BPA concentrations[135]. Most of these studies are cross-sectional, performed on a limited number of volunteers using spot urine BPA testing. Therefore, the long term effects of BPA could not be estimated. It has been reported that the presence of BPA in urine samples is positively associated with obesity in children, adolescents and adults, as well as with the promotion of obesity, especially the visceral type, increased metabolic risk through hyperinsulinemia, glucose intolerance, insulin resistance, elevated HbA1c and serum leptin levels and dyslipidemia[16,17,103,105,136-142]. Different research groups have reported a positive relationship between BPA levels and T2D[143-147]. It is worth noting that in some studies the obtained outcomes were independent of age, sex, ethnicity, body mass index, and serum cholesterol levels[104,148]. Furthermore, in a meta-analysis that included data from more than 41000 participants, detected BPA concentrations in urine and serum were positively associated with a risk for T2D[149]. In a recently performed cohort study with 1990 participants, the U-shaped curve reflected an association between serum BPA concentrations and risk for T2D[141]. Individuals with increased BPA concentrations and increased diabetes genetic risk score had increased fasting plasma glucose levels and risk for T2D as well[141]. In a longitudinal cohort study performed on more than 2300 adults of both sexes, repeated measurements were conducted in order to investigate the association of urinary BPA levels with glucose homeostasis parameters. The obtained results imply that BPA correlated with compromised glucose homoeostasis in women but not in men before the development of diabetes[150].

Prenatal BPA exposure was connected with an increased risk for lower birth weight, smaller size for gestational age as well as increased leptin and decreased adiponectin levels[151-154]. Significantly higher median urinary BPA levels were observed in children and adolescents with T1D when compared with healthy controls[102].

A limited number of studies have demonstrated BPA detection in adipose tissue, due to the invasive nature of the procedure and the complexity of the matrix. BPA was detected with high frequency (62%) in adipose tissue in children[155]. Moreover, obtained BPA levels in adipose tissue were much higher in children compared with adult women[156]. The levels of BPA in adipose tissue of adults were related to low GSH reductase activity and increased oxidized GSH, confirming that BPA triggers oxidative stress in human adipose tissue[157]. Regarding adipose tissue dysfunction, BPA serum levels were significantly higher in people with T2D in comparison with healthy controls; while a positive correlation with serum leptin levels, and a negative correlation with adiponectin was found in the group with diabetes, strongly suggesting that BPA may worsen diabetes and increase diabetes pathology[147].

AA

Overview

AA is an α,β-unsaturated carbonyl compound with electrophilic reactivity that has widespread applications in different industrial and laboratory processes[158]. In particular, AA is applied for the synthesis of polyacrylamide polymers used in water purification, sewage treatment, oil and sugar refinement, the production of soaps and cosmetics, varnishes, plastics, pesticides, adhesives, fibers, pharmaceuticals and textiles, and as a gel medium for electrophoresis methods in research laboratories[159-161]. AA is also found in cigarette smoke[162]. AA is the focus of scientific and public attention since 2002, when it was reported that it can be produced during the processing of certain foods. AA is formed as a result of a Milliard reaction when foods that contain asparagines and sugars are prepared at high temperatures (higher than 120 °C) under low moisture conditions[163-165]. More precisely, AA is formed during the browning of certain foods during frying, baking, grilling and roasting[159]. Hence, the main sources of AA in the diet are fried potatoes, breakfast cereals, cookies, crackers, crisps, bread, toast[166,167] and roasted coffee[168]. It is estimated that chronic average exposure to AA ranges from 0.5-1.9 μg/kg body weight per day in children, to 0.4-0.9 μg/kg body weight per day in adolescents, adults, and the elderly [169].

During detoxification processes, the majority of AA is conjugated to GSH, while less is metabolized to a genotoxic epoxide derivate glycidamide (GA) by the enzyme cytochrome P450 2E1 (CYP2E1)[170]. Genotoxic GA is more reactive than AA, and can produce DNA and Hb adducts[171]. The TDI for AA neurotoxicity is 40 μg/kg/d, while TDIs for cancer are 2.6 and 16 μg/kg/d for AA and GA, respectively [172]. Due to the adverse effects of AA on human health, the European Chemicals Agency ECHA has included AA on a list of candidate substances of very high concern that requires authorization from the European chemical regulation REACH (Registration, Evaluation, Authorization and Restriction of Chemicals)[159,173]. Several regulatory agencies provided different mitigation strategies for the prevention and reduction of AA formation in food[174-179].

AA and diabetes

Research status: Data about the association between low AA levels from diet and adverse health outcomes are still scarce and limited. To date, there have been only few attempts to investigate the impact of AA exposure on diabetes development. AA exposure disturbed the majority of redox status parameters *in vitro* in a β-cell line, Rin-5F, a validated β-cell model system[180]. Namely, AA exposure led to increased lipid peroxidation and nitric oxide (NO) production and a decrease in GSH content [180]. In addition, AA treatment affected the activity of antioxidant enzymes SOD and catalase (CAT), and the detoxifiying enzyme GSH S-transferase (GST) in pancreatic β-cells[180]. Formation of AA-GSH conjugates during detoxification could lead to GSH depletion and stimulation of GST activity in AA-exposed β-cells[180-182]. During metabolic processing, most AA is coupled to GSH *via* GST[158,183]. Elevated lipid peroxidation in pancreatic β-cells could be a result of GSH reduction[182]. AA exposure increased both the expression of inducible NO synthase (iNOS) and NO production in pancreatic β-cells, indicating induction of nitrosative stress[180]. Elevated iNOS and NO levels can cause β-cell dysfunction[184]. Decreased activity, but increased expression of SOD could be a consequence of the inactivation of redundant enzyme that is produced under conditions of high oxidative stress in AA-exposed pancreatic β-cells[185,186]. Upon AA exposure, resulting elevated NO levels reduced CAT activity in pancreatic β-cells[180,187]. *In vitro* metabolomics analysis revealed AA-induced glycolysis and gluconeogenesis alleviation characterized by diminished levels of glycolitic intermediates and a decreased rate of the tricarboxylic acid cycle[188]. Taken together, *in vitro* studies suggest that AA induces oxidative stress toxicity in β- cells and alters glucose metabolism.

In rats, AA exposure led to increased blood glucose levels and the development of histopathological changes in the islets[189]. In addition, a decreased β-cell and increased α-cell number was observed in

rats upon exposure to AA[190,191]. A similar pattern of islets remodeling characterized by α-cell expansion and β-cell reduction was detected in islets of both diabetic rats and humans[192-197]. These data are in line with the putative prodiabetic properties of AA. AA exposure altered expression of gluconeogenic enzymes in rats and mice, indicating the potential of AA to impair gluconeogenesis[189, 198]. Furthermore, AA affected the level of metabolites involved in the pentose phosphate pathway [199]. The pentose phosphate pathway is a significant component of glucose metabolism related to the development of T2D[200]. Taken together, these data demonstrate AA-induced disruption of glucose homeostasis. In addition, AA was shown to affect insulin-regulated IRS/PI3K/Akt/Foxo1 signaling pathways in rats[189]. Furthermore, AA exposure induced the expression of iNOS in rat pancreatic islets[180]. Increased iNOS expression impairs normal β-cell function and insulin secretion, and has been detected in both T1D and T2D[184]. In both *in vitro* and *in vivo* model systems, AA treatments reduced the expression of CYP2E1 in pancreatic β-cells[180]. CYP2E1 catalyzes biotransformation of AA to the genotoxic epoxide GA[170]. Reduction of CYP2E1 expression could be a protective mechanism in β-cells, in order to prevent the formation of the more toxic GA[180]. In addition, it has been shown that AA aggravates the diabetic condition in rodents[198,201,202]. Namely, AA worsens the histopathological features of liver and kidney lesions, blood biochemical parameters and redox status in diabetic rodents[198,201,202]. Diabetics are particularly vulnerable individuals, and more susceptible to environmental contaminants than the general population[186,198,203,204]. Collectively, *in vivo* studies in rodents indicate that AA exposure induces remodeling of pancreatic islets, impairs glucose metabolism and aggravates the overall diabetic state.

Potential mechanisms associated with diabetes: Based on the limited number of performed *in vitro* and *in vivo* studies, oxidative stress is the principle mechanism of AA-induced toxicity in pancreatic β-cells [180]. AA related impairment of both pentose phosphate pathway and insulin-regulated signaling is responsible for glucose metabolism disruption and development and aggravation of diabetes (Figure 2) [189,198,199].

Epidemiological evidence: Several epidemiological studies have revealed an association between AA intake and disorders of glucose metabolism[160,205,206]. In a Chinese adult population, a correlation between AA exposure and fasting plasma glucose levels was observed[160]. In line with these findings, data from the United States National Health and Nutrition Examination Survey (NHANES) 2003-2006 showed a significant correlation between high fasting plasma glucose levels and the concentration of HbGA adducts in the general adult population in the United States[205]. This study also reported that AA alters metabolic syndrome biomarkers[205]. Another NHANES study, 2003-2004, reported an association between AA exposure, decreased blood insulin levels and insulin resistance[206]. Subsequent NHANES surveys, 2005-2006 and 2013-2016, further confirmed these data and showed that Hb-AA adducts (HbAA) are linearly and inversely associated with the risk of diabetes development, whereas HbGA/HbAA nonlinearly and positively correlates with the prevalence of diabetes, indicating that HbAA and HbGA/HbAA are significantly associated with diabetes[169]. An association between HBAA adducts and AA intake was also detected in an adult Japanese population[207]. In addition, there is a link between prenatal dietary exposure to AA and the prevalence of obesity[208]. A large prospective study revealed a positive correlation between consumption of french fries and the risk for development of T2D in women[209]. French fries contain a high AA content: a standard portion contains approximately 30 μg of AA[165], indicating a significant contribution of AA to the development of T2D. These findings have been further confirmed by two prospective cohort studies, which showed an association between a high intake of ultra-processed foods and the risk of T2D[210,211]. Further epidemiological studies in other populations are required in order to confirm and elucidate the roles of AA exposure in the development of diabetes.

CONCLUSION

This paper summarizes important data, providing greater understanding of the diabetogenic effects of some PAEs and their metabolites, as well as BPA and AA. Risk assessment of these contaminants in mixtures of EDCs and the exact level of exposure associated with diabetes development over time remained unanswered. The effects of decreased exposure to phthalates, BPA, and AA through avoidance of specific packaging materials, or chemical reactions during food processing on glucose metabolism should also be addressed. Therefore, further prospective, well-designed studies with multiple measurements and longer follow-up, together with experimental studies, are required to completely understand the underlying mechanisms and confirm the causal association between PAEs, BPA, AA and diabetes outcomes. Diabetes is associated with serious complications, such as cardiovascular disease and stroke, chronic kidney disease, liver disease, neuropathy, retinopathy *etc*. Therefore, more effective prevention and treatment strategies are necessary. New strategies that advocate reduced exposure to food contaminants, while promoting increased physical activity and healthier nutritional choices, may be crucial for the prevention or delay of diabetes progression.

FOOTNOTES

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Targeting epicardial adipose tissue: A potential therapeutic strategy for heart failure with preserved ejection fraction with type 2 diabetes mellitus

Yu-Jiao Shi, Guo-Ju Dong, Ming Guo

Yu-Jiao Shi, Guo-Ju Dong, Ming Guo, Department of Cardiovascular Medicine, Xiyuan Hospital, Chinese Academy of Traditional Chinese Medicine, Beijing 100091, China

Corresponding author: Guo-Ju Dong, MD, PhD, Chief Physician, Department of Cardiovascular Medicine, Xiyuan Hospital, Chinese Academy of Traditional Chinese Medicine, No. 1 Xiyuan Playground, Haidian District, Beijing 100091, China. 13691393589@163.com

Abstract

Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous syndrome with various comorbidities, multiple cardiac and extracardiac pathophysiologic abnormalities, and diverse phenotypic presentations. Since HFpEF is a heterogeneous disease with different phenotypes, individualized treatment is required. HFpEF with type 2 diabetes mellitus (T2DM) represents a specific phenotype of HFpEF, with about 45%-50% of HFpEF patients suffering from T2DM. Systemic inflammation associated with dysregulated glucose metabolism is a critical pathological mechanism of HFpEF with T2DM, which is intimately related to the expansion and dysfunction (inflammation and hypermetabolic activity) of epicardial adipose tissue (EAT). EAT is well established as a very active endocrine organ that can regulate the pathophysiological processes of HFpEF with T2DM through the paracrine and endocrine mechanisms. Therefore, suppressing abnormal EAT expansion may be a promising therapeutic strategy for HFpEF with T2DM. Although there is no treatment specifically for EAT, lifestyle management, bariatric surgery, and some pharmaceutical interventions (anti-cytokine drugs, statins, proprotein convertase subtilisin/kexin type 9 inhibitors, metformin, glucagon-like peptide-1 receptor agonists, and especially sodium-glucose cotransporter-2 inhibitors) have been shown to attenuate the inflammatory response or expansion of EAT. Importantly, these treatments may be beneficial in improving the clinical symptoms or prognosis of patients with HFpEF. Accordingly, well-designed randomized controlled trials are needed to validate the efficacy of current therapies. In addition, more novel and effective therapies targeting EAT are needed in the future.

Key Words: Epicardial adipose tissue; Heart failure with preserved ejection fraction; Type 2 diabetes mellitus; Inflammation; Anti-hyperglycemic drugs; Sodium-glucose

cotransporter-2 inhibitors

Core Tip: Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous syndrome requiring individualized treatment depending on phenotypic differences. HFpEF with type 2 diabetes mellitus is strongly associated with the expansion, inflammation, and hypermetabolic activity of epicardial adipose tissue (EAT). Thus, targeting EAT may be a promising therapeutic strategy for HFpEF with type 2 diabetes mellitus. Lifestyle management, bariatric surgery, and certain drugs may suppress the accumulation of EAT and improve the clinical symptoms and prognosis of HFpEF. More studies are required to validate the efficacy of current treatments and to develop new effective therapies.

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF), a systemic and heterogeneous syndrome, is characterized by various comorbidities (mainly diabetes mellitus, hypertension, and metabolic syndrome), multiple cardiac and extracardiac pathophysiologic abnormalities, and diverse phenotypic presentations[1]. HFpEF is a growing public health challenge, which currently accounts for approximately half of HF cases, and its prevalence continues to rise due to an aging population and the increasing burden of comorbidities[2]. Additionally, HFpEF is associated with poor prognosis, with a 5-year mortality rate of up to 75%[3]. Standardized and effective interventions are lacking due to the complex pathophysiological underpinnings and clinical heterogeneity of HFpEF[4]. It may, however, be beneficial to halt disease progression and thus improve prognosis by providing individualized treatment based on phenotypic differences[4].

Type 2 diabetes mellitus (T2DM) is a substantial risk factor for the emergence and progression of HFpEF, and approximately 45%-50% of HFpEF cases suffer from T2DM, a specific phenotype of HFpEF [5,6]. Systemic inflammation related to glucose metabolism disorders is accepted as a critical pathological mechanism of HFpEF with T2DM, which is responsible for the expansion and dysfunction (inflammation and hypermetabolic activity) of epicardial adipose tissue (EAT)[7]. EAT, a metabolically active visceral fat depot, can regulate the pathophysiological processes of HFpEF with T2DM through the paracrine and endocrine mechanisms[8]. Thus, inhibiting the accumulation of EAT may be a promising therapeutic strategy for HFpEF with T2DM. At present, lifestyle management, bariatric surgery, and some medications may contribute to reducing the inflammation response or accumulation of EAT, despite the fact that there is no available treatment for EAT. Notably, these interventions may attenuate pathological changes and improve the prognosis in patients with HFpEF.

Currently, a comprehensive review is lacking discussing the pathogenesis of EAT-mediated HFpEF with T2DM and therapies to inhibit EAT expansion. In this review, we evaluated the role of EAT in the development of HFpEF with T2DM and discussed current therapies to attenuate EAT expansion as well as future therapeutic perspectives.

ANATOMY, PATHOLOGY AND PATHOPHYSIOLOGY OF EAT

Anatomy of EAT

EAT represents the local visceral fat depot of the heart, located between the myocardium and the visceral pericardium[9] (Figure 1). Under healthy circumstances, EAT accounts for approximately 20% of the total heart weight and covers 80% of the cardiac surface[10,11]. In adults, EAT typically surrounds the coronary arteries and their major epicardial branches, mainly concentrated in the interventricular and atrioventricular grooves, with lesser amounts covering the atria, the free wall of the right ventricle, and the apex[9]. Interestingly, EAT is anatomically and functionally contiguous with the myocardium because of the shared microcirculation and the absence of muscle fascia, which may facilitate the local interaction of EAT with the myocardium and coronary arteries through vasocrine or paracrine cross-talk [12]. Microscopically, EAT consists typically of adipocytes specialized in energy storage but also includes inflammatory cells (mainly macrophages and mast cells), immune cells, stromovascular cells,
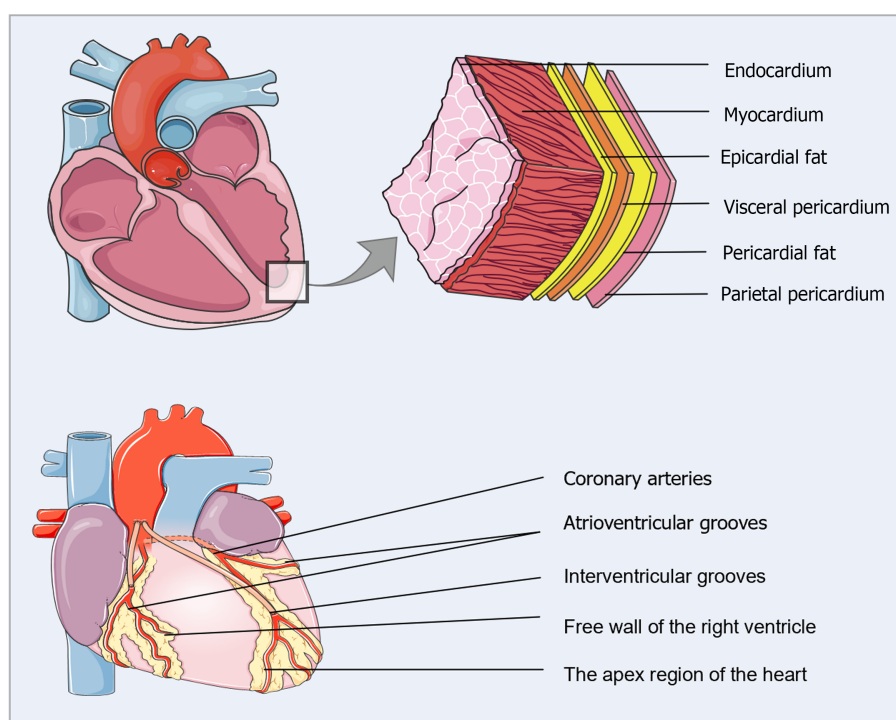
Figure 1 Anatomical location of epicardial adipose tissue. Epicardial adipose tissue (EAT) is situated between the myocardium and the visceral pericardium. In normal adults, EAT usually accompanies the coronary arteries and their major epicardial branches, mainly concentrated in the interventricular and atrioventricular grooves, with lesser amounts covering the atria, the free wall of the right ventricle, and the apex.

and ganglia in normal adults. In pathological states, however, numerous inflammatory cell aggregates and abnormal expansion of the microvascular network are present in the EAT[13].

Physiology of EAT

EAT acts as a shock absorber, protecting coronary arteries from excessive distortion and compression during the contraction of the adjacent myocardium[14]. EAT has a greater capacity to release and uptake free fatty acids (FFA) compared to other visceral fat depots. The myocardium metabolizes FFAs from the coronary arterial blood, which is shared with the contiguous EAT. FFA oxidation is responsible for almost 50%-70% of the energy production in the heart[15]. Accordingly, EAT might serve as a physiological buffer to protect the myocardium from excessive fatty acid levels and as a direct energy source to provide FFA under increased metabolic demand. Moreover, EAT expresses uncoupling protein-1 (UCP1), a thermogenic protein located in the inner membrane of mitochondria. UCP1 uncouples oxidative phosphorylation from ATP synthesis, ultimately dissipating energy as heat[16]. EAT might, therefore, provide direct heat to the myocardium and protect the heart under unfavorable hemodynamic conditions.

Pathophysiology of EAT

EAT has been widely established as a remarkably active endocrine organ that secretes various bioactive molecules, such as cytokines, adipokines, and chemokines, that can exert protective or detrimental effects depending on the local microenvironmental situation[17]. EAT can, therefore, locally modulate the adjacent myocardium and coronary arteries through the vasocrine or paracrine secretion of these bioactive molecules[12]. Physiologically, EAT mainly releases anti-inflammatory adipocytokines, such as adiponectin, adrenomedullin, omentin, and interleukin-10 (IL-10), which contribute to cardioprotection and anti-atherosclerosis[14]. In contrast, adipocytes enlarge and produce high quantities of FFAs under pathological conditions, triggering EAT expansion, localized hypoxia, and the infiltration of macrophages, ultimately resulting in a chronic inflammatory response[8]. Subsequently, numerous proinflammatory adipokines are produced and accumulated, including IL-6, tumor necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1, leptin, resistin, and serglycin, which aggravate local inflammation, thereby affecting the heart and coronary arteries[12].

CONTRIBUTIONS OF EAT TO HFPEF WITH T2DM

EAT in the pathophysiology of HFpEF with T2DM

Dysregulated glucose metabolism is a fundamental clinical characteristic of T2DM and is strongly connected with the aberrant accumulation of EAT[18-20]. As reported in Table 1, EAT thickness over the right ventricular free wall, EAT volume, or EAT area were significantly higher in patients with impaired fasting glucose, insulin resistance, or T2DM than in control subjects[21-39]. A meta-analysis of nine studies by Li *et al*[40] confirmed a positive correlation between the presence of T2DM and EAT expansion. Eventually, increased EAT deposition interacts directly with the heart through mechanical and metabolic mechanisms, leading to myocardial fibrosis, cardiomyocyte stiffness, and left ventricular (LV) diastolic dysfunction, which are the essential pathological features of HFpEF (Figure 2).

In terms of machinery, increased EAT occupies a large space in the cardiac fossa and applies a compressive contact force on the heart, resulting in pericardial restrain, increased ventricular filling pressures, and LV diastolic dysfunction. A meta-analysis of 11 studies showed that increasing EAT was independently associated with LV diastolic dysfunction even after adjusting for age, sex, and measures of adiposity[41]. In patients with T2DM, Christensen *et al*[27] and Song *et al*[42] substantiated the deleterious effect of increased EAT on LV global longitudinal strain and LV diastolic function assessed by peak velocity during early diastole (E)/peak velocity during atrial contraction (A) ratio, early diastolic mitral annular velocity (e'), and E/e' ratio.

In terms of metabolism, EAT enlargement is linked to the buildup of FFAs and lipid metabolites[43], which induce myocardial lipotoxicity and in turn contribute to excessive oxidative stress, endoplasmic reticulum stress, and mitochondrial dysfunction, ultimately causing LV diastolic dysfunction[44]. Furthermore, excessive cardiomyocyte lipid deposits may lead to cardiac steatosis, which has been demonstrated to be an early marker of diabetic heart disease and is independently associated with LV diastolic function[45-47]. Simultaneously, hypertrophic adipocytes and activated macrophages exhibit increased production of proinflammatory adipocytokines and chemokines in EAT. These proinflammatory factors cause local inflammation, excessive oxidative stress, microvascular and endothelial dysfunction, and extracellular matrix deposition through vasocrine or paracrine mechanisms, resulting in cardiomyocyte stiffness, myocardial fibrosis, and subsequent LV diastolic dysfunction[8,9].

Relationship between increased EAT and clinical characteristics of HFpEF

As shown in Table 2, EAT expansion is closely related to severe pathologic changes, clinical manifestations, and long-term prognosis in individuals with HFpEF[48-55]. According to research by van Woerden *et al*[48] and Pugliese *et al*[54], enlarged EAT is linked to increased plasma myocardial injury markers. Wang *et al*[49] found that the EAT volume was positively correlated with elevated inflammatory markers (C-reactive protein), LV hypertrophy (LV mass index), and LV diastolic dysfunction (E/e' ratio and tricuspid regurgitation velocity). Venkateshvaran *et al*[50] confirmed that higher EAT was linked not only to LV hypertrophy and diastolic dysfunction but also to endothelial dysfunction. Koepp *et al*[51] showed that thickened EAT was associated with elevated cardiac filling pressures, pulmonary hypertension, and pericardial constraint. Additionally, some studies have confirmed that increased EAT may lead to decreased exercise tolerance or quality of life[50-54]. Importantly, EAT thickening was correlated with a 1.12-fold increased risk of the composite endpoint of death and HF hospitalization after 21 mo of follow-up, according to Pugliese *et al*[54]. After 24 mo of follow-up, van Woerden *et al*[55] confirmed that EAT expansion increased the risk of all-cause mortality, HF hospitalization, and the composite endpoint.

CURRENT INTERVENTIONS TARGETING EAT AND FUTURE THERAPEUTIC PERSPECTIVES IN HFPEF WITH T2DM

EAT plays an important role in the development and progression of HFpEF with T2DM and is strongly associated with an increased risk of adverse outcomes. Therefore, alleviating EAT expansion may be a promising therapeutic strategy. Although no treatment is available specifically for EAT, lifestyle management, bariatric surgery, and medications (Table 3) including anti-hyperlipidemia, anti-cytokines, and anti-hyperglycemia have been demonstrated to reduce the inflammation response or expansion of EAT and appear to be beneficial for HFpEF (Figure 3).

Non-pharmacological interventions

In diabetic and obese patients, lifestyle modifications (including a low-calorie diet and exercise training) and bariatric surgery can reduce EAT levels. Twenty severely obese patients were shown to have a 32% reduction in EAT thickness and alleviation in LV hypertrophy and diastolic dysfunction after 6 mo of calorie restriction with moderate exercise[56]. Serrano-Ferrer *et al*[57] confirmed that exercise training significantly reduced EAT thickness and serum TNF-α, increased lipocalin, and improved LV myocardial strain and strain rate. A study by Honkala *et al*[58] reported that 2 wk of continuous exercise

Table 1 Epicardial adipose tissue expansion in patients with glucose metabolism disorders

Ref.	Participants, *n*	Amount of EAT in the observation group	Amount of EAT in the control group	*P* value
EAT thickness (mm) measured by echocardiography thickness on the right ventricular free wall				
Baloglu *et al*[21], 2019	T2DM patients: 128; healthy controls: 32	3.53 ± 0.79	4.64 ± 1.39	< 0.001
Akbas *et al*[22], 2014	T2DM patients: 156; healthy controls: 50	4.66 ± 1.50	3.91 ± 1.60	0.005
Chen *et al*[23], 2017	T2DM patients: 167; healthy controls: 82	4.00 (3.00-5.00)	2.00 (1.00-3.00)	< 0.001
Philouze *et al*[24], 2017	T2DM patients: 44; healthy controls: 35	6.40 ± 1.70	3.30 ± 1.10	< 0.001
Cetin *et al*[25], 2013	T2DM patients: 139; age- and sex-matched controls: 40	6.00 ± 1.50	4.42 ± 1.00	< 0.001
Yafei *et al*[26], 2019	T2DM patients: 76; age- and sex-matched controls: 30	6.23 ± 1.27	4.60 ± 1.03	< 0.001
Christensen *et al*[27], 2019	T2DM patients: 770; age- and sex-matched controls: 234	4.60 ± 1.80	3.40 ± 1.20	< 0.0001
Wang *et al*[28], 2017	T2DM with duration ≤ 10 yr: 35; T2DM with duration > 10 yr: 33	4.47 ± 1.90	5.45 ± 1.40	< 0.05
Altin *et al*[29], 2016	Patients with IR: 113; age- and sex-matched controls: 112	7.34 ± 1.96	5.22 ± 1.75	< 0.001
Iacobellis *et al*[30], 2008	Patients with IFG: 65; non-diabetic controls: 50	Males: 8.00 ± 3.00	6.00 ± 2.00	< 0.001
		Females: 7.10 ± 4.00	5.80 ± 3.00	
EAT volume (cm^3) measured by computed tomography				
Wang *et al*[31], 2008	T2DM patients: 49; non-diabetic controls: 78	166.1 ± 60.6	123.4 ± 41.8	< 0.0001
Akyürek *et al*[32], 2014	T2DM patients: 93; non-diabetic controls: 85	40.1 ± 23.9	16.9 ± 7.7	< 0.001
Gullaksen *et al*[33], 2019	T2DM patients: 44; non-diabetic controls: 59	119.0 ± 49.0	86.0 ± 40.0	< 0.001
Groves *et al*[34], 2014	T2DM patients: 92; non-diabetic controls: 59	118.6 ± 43.0	70.0 ± 44.0	< 0.0001
Versteylen *et al*[35], 2012	Patients with IFG: 118; non-diabetic controls: 209	92.0 ± 39.0	75.0 ± 34.0	< 0.001
EAT volume (cm^3) or area (cm^2) measured by cardiac magnetic resonance				
Huang *et al*[36], 2022	T2DM with duration ≤ 5 yr: 56; T2DM with duration > 5 yr: 57	48.4 ± 13.4 cm^3	58.4 ± 17.3 cm^3	< 0.001
Evin *et al*[37], 2016	T2DM patients: 20; healthy controls: 19	135.0 ± 31.0 cm^3	90.0 ± 30.0 cm^3	< 0.001
Al-Talabany *et al*[38], 2018	T2DM patients: 54; non-diabetic controls: 29	13.5 ± 3.5 cm^2	11.8 ± 4.1 cm^2	< 0.05
Rado *et al*[39], 2019	Prediabetes patients: 100; healthy controls: 200	9.2 cm^2	7.7 cm^2	< 0.001

EAT: Epicardial adipose tissue; IFG: Impaired fasting glucose; IR: Insulin resistance; T2DM: Type 2 diabetes mellitus.

training resulted in decreased EAT volume and myocardial triglyceride levels and improved aerobic exercise tolerance and insulin sensitivity in 16 patients with T2DM. A meta-analysis including five studies confirmed that exercise training reduced epicardial fat deposition[59].

Several studies have reported that bariatric surgery substantially reduces the accumulation of EAT in patients[60-64]. Gaborit *et al*[62] found a 27% reduction in EAT volume in obese patients at a 6-mo follow-up after bariatric surgery. In addition, individuals with HFpEF appear to benefit from lifestyle changes and bariatric surgery in terms of improved microvascular and endothelial dysfunction, left ventricular remodeling and diastolic dysfunction, exercise tolerance, and quality of life[65-68]. Thus, lifestyle modification and bariatric surgery may alleviate the abnormal expansion of EAT in HFpEF patients with obesity and T2DM and improve LV diastolic function and clinical symptoms. Nevertheless, further research is required to determine whether it can improve the prognosis of patients.

Table 2 Relationship between increased epicardial adipose tissue and clinical characteristics of heart failure with preserved ejection fraction

Ref.	Participants, *n*	Imaging method	Relationship between increased EAT and clinical characteristics of HFpEF		
			Pathological changes	Clinical manifestations	Prognosis
van Woerden *et al* [48], 2018	64 HF patients with LVEF > 40%	CMR	Myocardial injury: increased creatine kinase-MB and TnT	Decreased quality of life (KCCQ score)	
Wang *et al*[49], 2022	53 HF patients with LVEF > 50%	CMR	Inflammation: increased CRP; LV hypertrophy: increased LVmass index; LV diastolic dysfunction: increased E/e' and tricuspid regurgitation velocity		
Venkateshvaran *et al* [50], 2022	182 HF patients with LVEF > 50%	Echo	Inflammation; endothelial dysfunction; LV hypertrophy: increased LV septal wall thickness; LV diastolic dysfunction: increased E peak deceleration time	Decreased quality of life (KCCQ score)	
Koepp *et al*[51], 2020	169 HF patients with LVEF > 50%	Echo	Increased cardiac filling pressures, pulmonary hypertension, and pericardial restraint	Decreased exercise capacity (VO$_2$, AVO$_2$ diff)	
Haykowsky *et al* [52], 2018	100 HF patients with LVEF > 50%	CMR		Decreased exercise capacity (VO$_2$, 6-min walk test, leg power)	
Gorter *et al*[53], 2020	75 HF patients with LVEF > 45%	Echo		Decreased exercise capacity (VO$_2$)	
Pugliese *et al*[54], 2021	188 HF patients with LVEF > 50%	Echo	Myocardial injury: increased TnT; inflammation: increased CRP	Decreased exercise capacity (peak VO$_2$ and AVO$_2$ diff)	Increased risk of the composite endpoint of HF hospitalization and cardiovascular deaths
van Woerden *et al* [55], 2022	105 HF patients with LVEF > 40%	CMR			Increased risk of HF hospitalization, all-cause death, and the composite endpoint

AVO$_2$ diff: Non-invasive arterial-venous oxygen content difference; CMR: Cardiac magnetic resonance; CRP: C-reactive protein; EAT: Epicardial adipose tissue; Echo: Echocardiography; E/e': Peak velocity during early diastole/early diastolic mitral annular velocity; HF: Heart failure; HFpEF: Heart failure with preserved ejection fraction; KCCQ: Kansas City cardiomyopathy questionnaire; LV: Left ventricular; LVEF: Left ventricular ejection fraction; MB: Myocardial band; TnT: Troponin T; VO$_2$: Peak oxygen consumption.

Pharmacological interventions

Anti-cytokine drugs: Inflammation is an essential driver of abnormal EAT expansion. Theoretically, anti-cytokine drugs (anti-IL-1 and anti-IL-6, *etc*) can interfere with the pathophysiological process of EAT expansion and may eventually decrease EAT accumulation. Unfortunately, there are no relevant studies to confirm this. Furthermore, anti-cytokine drugs, particularly IL-1 blockade, have shown cardioprotective effects in many cardiovascular diseases[69]. Nevertheless, few clinical studies have examined their effects on HFpEF, and the results are inconsistent. The D-HART trial showed that a 14-d intervention with anakinra, an IL-1 blocker, significantly reduced the systemic inflammatory response and improved aerobic exercise capacity in individuals with HFpEF (*n* = 12)[70]. Contrarily, the D-HART 2 trial found that anakinra intervention for 12 d failed to improve exercise capacity in patients with HFpEF (*n* = 21)[71]. Therefore, whether anti-cytokine drugs reduce EAT deposition has not been confirmed in clinical investigations, and their role in HFpEF with T2DM requires validation in standardized randomized controlled trials.

Anti-hyperlipidemic drugs: Statins are 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors that can significantly reduce endogenous cholesterol production by inhibiting the rate-limiting enzyme in cholesterol synthesis[72]. As the anti-inflammatory effects have been established, researchers have begun to explore the role of statins in EAT in the last decade. According to Parisi *et al*[73], statin therapy dramatically decreased EAT thickness and EAT-secreted inflammatory mediators in individuals with aortic stenosis. In patients who successfully underwent percutaneous coronary intervention, Park *et al* [74] demonstrated that atorvastatin (20 mg/d) reduced EAT thickness more significantly than simvastatin/ezetimibe (10/10 mg/d). Soucek *et al*[75] confirmed that substantial reductions in EAT were associated with intensive atorvastatin therapy (80 mg/d) in atrial fibrillation patients undergoing pulmonary vein isolation. A study by Alexopoulos *et al*[76] showed that intensive treatment (ator-vastatin, 80 mg/d) was more successful in inducing EAT reduction than moderate-intensity treatment

Table 3 Pharmacological interventions targeting epicardial adipose tissue

Ref.	Imaging method	Participants, *n*	Intervention method and duration	Change of EAT	Other findings
Park *et al*[74], 2010	Echo	145 coronary artery stenosis patients	Atorvastatin: *n* = 82, 20 mg/d; simvastatin: *n* = 63, 10 mg/d; for 6-8 mo	Atorvastatin decreased EAT thickness (0.47 ± 0.65 mm) more than simvastatin (EAT 0.12 ± 0.52 mm, *P* = 0.001)	Decreased TC, TG, and LDL-C
Soucek *et al* [75], 2015	CT	38 atrial fibrillation patients	Atorvastatin: 80 mg/d, for 3 mo	EAT volume decreased from 86.9 (64.1-124.8) mL to 92.3 (62.0- 133.3) mL (*P* < 0.05)	Decreased CRP, TC, and LDL-C
Alexopoulos *et al*[76], 2013	CT	420 hyperlipidemic post-menopausal women	Atorvastatin: *n* = 194, 80 mg/d; pravastatin: *n* = 226, 40 mg/d; for 12 mo	Atorvastatin decreased EAT volume (3.38%) more than pravastatin (0.83%, *P* = 0.025)	Decreased TC, TG, and LDL-C
Rivas Galvez *et al*[78], 2020	Echo	41 patients treated with PCSK9 inhibitors	Evolocumab: *n* = 16; alirocumab: *n* = 8; twice in 6 mo	EAT thickness decreased by 20.39% (*P* = 0.0001).	Decreased BMI, TC, and LDL-C
Iacobellis *et al* [82], 2017	Echo	41 patients T2DM	Metformin: 500 mg-1000 mg, twice daily, for 6 mo	EAT thickness changed from 7.4 ± 1.6 mm to 7.5 ± 1.5 mm and 6.9 ± 1.3 mm at 3 and 6 mo, respectively	Decreased BMI
Ziyrek *et al* [83], 2019	Echo	40 T2DM patients	Metformin: 1000 mg, twice daily, for 3 mo	EAT thickness decreased from 5.07 ± 1.33 mm to 4.76 ± 1.32 mm (*P* < 0.001)	
Iacobellis *et al* [84], 2020	Echo	51 T2DM patients	Metformin: 500 mg-1000 mg, twice daily, for 6 mo	EAT thickness decreased from 8.0 ± 2.5 mm to 7.4 ± 2.5 mm and 7.5 ± 2.4 mm at 3 and 6 mo, respectively (compared with baseline *P* < 0.016)	
Moody *et al* [90], 2014	CMR	12 T2DM patients	Pioglitazone: 15 mg/d, for 2 wk, then increase to 45 mg/d, for 22 wk	EAT area decreased from 15.3 ± 3.9 cm^2 to 14.0 ± 3.9 cm^2 (*P* = 0.03)	Decreased paracardial adipose tissue; improved left ventricular diastolic function
Lima-Martínez *et al*[94], 2015	Echo	26 T2DM patients	Combination of sitagliptin (50 mg) and metformin (1000 mg), twice daily, for 24 wk	EAT thickness reduction of 15% (*P* = 0.001)	
van Eyk *et al* [99], 2019	CMR	22 T2DM patients	Liraglutide: 0.6 mg/d gradually increased to 1.8 mg/d in 2 wk, for 26 wk	EAT area reduction of 0 ± 2 cm^2	Decreased visceral fat volume
Bizino *et al* [100], 2020	CMR	23 T2DM patients	Liraglutide: 0.6 mg/d gradually increased to 1.8 mg/d in 2 wk, 26 wk	EAT area reduction of 1.1 ± 6.0 cm^2	Decreased body weight and subcutaneous fat
Iacobellis *et al* [82], 2017	Echo	54 T2DM patients	Combination of liraglutide (increased to 1.8 mg/once daily) and metformin (1000 mg, twice daily), for 12 wk	EAT thickness reduction of 29% and 36% at 3 and 6 mo, respectively	Decreased BMI and HbA1c
Zhao *et al* [101], 2021	Echo	21 T2DM patients	Liraglutide: 0.6 mg/d gradually increased to 1.2 mg/d in 3-5 d, for 3 mo	EAT decreased from 5.00 (5.0-7.0) mm to 3.95 ± 1.43 mm (*P* < 0.001)	Decreased weight, HbA1c, TC, TG, and LDL-C
Dutour *et al* [102], 2016	CMR	22 T2DM patients	Exenatide: 5 mg twice daily, for 4 wk, then increase to 10 mg twice daily, for 22 wk	EAT volume reduction of 8.8 ± 2.1%	Decreased weight, HbA1c, and hepatic triglyceride content
Morano *et al* [103], 2015	Echo	25 T2DM patients	Combination of exenatide (5 mg twice daily, for 1 mo, and then increase to 10 mg twice daily, for 2 mo) and liraglutide (1.2 mg/d), for 3 mo	EAT thickness decreased from 9.4 ± 1.6 mm to 8.0 ± 1.9 mm (*P* = 0.003)	Decreased MRI; improved renal resistive index
Iacobellis *et al* [104], 2020	Echo	6 T2DM patients	Semaglutide: *n* = 30, 1 mg weekly; dulaglutide: *n* = 30, 1.5 mg weekly; for 12 wk	EAT thickness reduction of 20% in both semaglutide and dulaglutide groups	Decreased BMI and HbA1c
Requena *et al* [108], 2021	CMR	84 non-diabetic patients with HFrEF	Empagliflozin: 10 mg/d, for 6 mo	EAT volume reduction of 5.14 mL, *P* < 0.05	Decreasing subcutaneous fat and matrix volume
Ardahanlı *et al* [109], 2021	Echo	37 T2DM patients	Empagliflozin: 10 mg/d, for 6 mo	EAT thickness decreased from 7.6 ± 1.7 mm to 6.7 ± 1.3 mm (*P*	Decreased BMI, waist circum-ference, HbA1c, uric acid,

				< 0.001)	systolic and diastolic blood pressure, and carotid intima-media thickness
Iacobellis *et al* [84], 2020	Echo	51 T2DM patients	Combination of dapagliflozin (5 to 10 mg/d) and metformin (500 to 1000 mg, twice daily), for 24 mo	EAT thickness decreased by 15% from baseline to 12 wk and 20% after 24 wk (compared with baseline $P < 0.01$)	Decreased weight and HbA1c
Sato *et al*[110], 2018	CT	20 T2DM patients	Dapagliflozin: 10 mg/d, for 6 mo	EAT volume reduction of 16.4 ± 8.3 mL ($P < 0.05$)	Decreased HbA1c, TNF-α, TG, insulin resistance, and left atrial dimension
Sato *et al*[111], 2020	CT	18 T2DM patients with coronary artery disease	Dapagliflozin: 5 mg/d, for 6 mo	EAT volume reduction of 15.2 ± 12.8 mL ($P < 0.05$)	Decreased HbA1c, TNF-α, and insulin resistance
Braha *et al* [112], 2021	CT	52 T2DM patients	Dapagliflozin: 10 mg/d, for 6 mo	EAT volume reduction of 17.1% ($P < 0.001$)	Decreased BMI, triglyceride glucose index, and HbA1c
Yagi *et al*[113], 2017	Echo	13 T2DM patients	Canagliflozin: 100 mg/d, for 6 mo	EAT thickness decreased from 9.3 ± 2.5 to 8.1 ± 2.3 mm ($P < 0.01$) and to 7.3 ± 2.0 mm ($P < 0.001$) at 3 mo and 6 mo, respectively	Decreased BMI
Fukuda *et al* [114], 2017	CMR	9 T2DM patients	Ipragliflozin: 50 mg/d, 12 wk	EAT volume decreased from 102 (79-126) mL to 89 (66-109) mL ($P = 0.008$)	Decreased weight, BMI, HbA1c, TG, leptin, fasting plasma glucose, and insulin resistance
Bouchi *et al* [115], 2017	CMR	19 T2DM patients	Luseogliflozin: 2.5-5.0 mg/d for 12 wk	EAT volume decreased from 117 (96-136) mL to 111 (88-134) mL ($P = 0.048$)	Decreased weight, BMI, systolic and diastolic blood pressure, HbA1c, fasting plasma glucose, insulin resistance, and CRP
Gaborit *et al* [116], 2021	CMR	26 T2DM patients	Empagliflozin: 10 mg/d, 12 wk	EAT volume decreased from 108.5 ± 31.8 mL to 106.9 ± 31.8 mL ($P = 0.09$)	Decreased BMI, TG, HbA1c, fasting blood glucose, liver fat content, and visceral fat volume

BMI: Body mass index; CMR: Cardiovascular magnetic resonance; CRP: C-reactive protein; CT: Computed tomography; EAT: Epicardial adipose tissue; Echo: Echocardiography; HbA1c: Glycosylated hemoglobin; HFrEF: Heart failure with reduced ejection fraction; LDL-C: Low-density lipoprotein cholesterol; MRI: Magnetic resonance imaging; PCSK9: Proprotein convertase subtilisin/kexin type 9; T2DM: Type 2 diabetes mellitus; TC: Total cholesterol; TG: Triglycerides; TNF-α: Tumor necrosis factor-α.

(pravastatin, 40 mg/d) in hyperlipidemic post-menopausal women.

Furthermore, proprotein convertase subtilisin/kexin type 9 (PCSK9), part of the EAT secretome, is involved in EAT-induced inflammation[77]. Therefore, PCSK9 inhibitors, a new class of lipid-lowering drugs, may inhibit the abnormal expansion of EAT. A non-randomized cohort of 24 patients reported a 20.39% reduction in EAT thickness after 6 mo of PCSK9 inhibitor treatment (evolocumab or alirocumab) [78]. In recent years, statin therapy has been reported to considerably reduce mortality in patients with HFpEF, possibly associated with a reduction in the inflammatory response or accumulation of EAT[79, 80]. Thus, hypolipidemic medicines may attenuate aberrant EAT expansion and be advantageous in diabetic HFpEF, and well-designed randomized controlled trials are still needed to validate this.

Anti-hyperglycemic drugs: Metformin, an oral anti-hyperglycemic drug for patients with T2DM, lowers blood glucose levels by decreasing hepatic glucose production (gluconeogenesis) and improves insulin sensitivity by increasing peripheral glucose uptake and utilization[81]. In recent years, several studies have begun to explore its impacts on EAT, as its positive effects on reducing body weight and fat composition have been revealed. Iacobellis *et al*[82] showed that metformin treatment (500-1000 mg, twice daily) for 3-6 mo failed to reduce EAT thickness in patients with T2DM. In contrast, Ziyrek *et al* [83] found a significant reduction of EAT thickness after 3 mo of metformin monotherapy (1000 mg, twice daily) in individuals with T2DM. After increasing the sample size, Iacobellis *et al*[84] also discovered that metformin slightly reduced EAT thickness. Additionally, metformin treatment decreased mortality in HFpEF patients and improved LV hypertrophy and diastolic dysfunction[85,86]. Unfortunately, studies on the effects of metformin on EAT accumulation are scarce and controversial, and future research is needed to generate robust evidence.

Thiazolidinediones (TZDs), which are peroxisome proliferator-activated receptor gamma (PPAR- γ) agonists, can enhance insulin sensitivity by activating peroxisome proliferator-activated receptor gamma[87]. As a result, it reduces the secretion of proinflammatory cytokines in the visceral fat depots and thereby can inhibit the abnormal enlargement of EAT[88]. Pioglitazone, a member of TZDs, was shown to significantly reduce EAT inflammatory markers (IL-6, TNF-α, resistin, and matrix metalloproteinase-9) and increase adiponectin in patients with coronary artery disease and metabolic syndrome
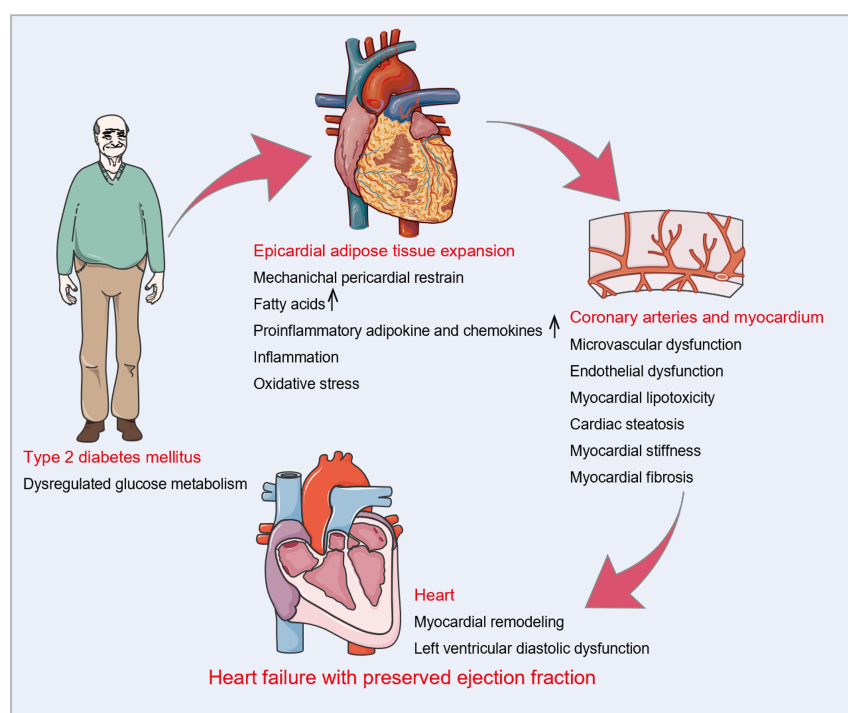
Figure 2 Epicardial adipose tissue in the pathophysiology of heart failure with preserved ejection fraction with type 2 diabetes mellitus.
Dysregulated glucose metabolism is intimately related to the expansion of epicardial adipose tissue (EAT). Increased EAT deposition interacts directly with the heart through mechanical and metabolic mechanisms. Mechanically, EAT expansion may directly contribute to pericardial restrain, resulting in left ventricular (LV) diastolic dysfunction. Metabolically, EAT enlargement is linked to the buildup of free fatty acids, which may induce myocardial lipotoxicity and cardiac steatosis. Simultaneously, hypertrophic adipocytes and activated macrophages secrete numerous proinflammatory adipocytokines and chemokines in EAT. Subsequent local inflammation, excessive oxidative stress, microvascular and endothelial dysfunction, and myocardial stiffness and fibrosis ultimately lead to LV remodeling and diastolic dysfunction.

[89]. According to Moody *et al*[90], pioglitazone treatment was linked to a 9% reduction in EAT area and improvement in LV diastolic function in patients with T2DM, and there was a significant negative correlation between EAT and LV diastolic function. However, TZDs may cause serious cardiovascular adverse effects, especially HF[91,92]. As a result, the clinical use of TZDs in the treatment of HFpEF is limited due to their potential to exacerbate HF.

Dipeptidyl peptidase 4 (DPP-4) inhibitors improve glucose-dependent insulin secretion by increasing bioactive incretins, which inhibit glucagon release and then promote insulin production to decrease blood glucose levels[93]. Only a single-group pre-post study by Lima-Martínez *et al*[94] showed that 26 overweight patients with T2DM had a 15% reduction in EAT thickness after 6 mo of treatment with a combination of metformin and sitagliptin, a DPP-4 inhibitor. Unfortunately, there is a lack of research on regulating EAT using DPP-4 inhibitors alone. Therefore, relevant studies still need to support whether DPP-4 inhibitors can reduce EAT accumulation. In addition, it is controversial whether an increased risk of HF is associated with DPP-4 inhibitors[95].

Glucagon-like peptide-1 receptor agonists (GLP1-RAs) comprise a novel anti-diabetic drug class that maintains glucose homeostasis by stimulating glucose-dependent insulin secretion, suppressing glucagon release, and inhibiting gastric emptying[96]. Previous studies reported the presence of GLP-1R in EAT with mRNA and protein expression, and targeting GLP-1R in EAT can reduce local adipogenesis, enhance fat utilization, and drive brown fat differentiation[97,98]. According to research by van Eyk *et al*[99] and Bizino *et al*[100], liraglutide reduced visceral or subcutaneous fat but failed to reduce EAT accumulation in T2DM. Five investigations, however, demonstrated that liraglutide[82,101-103], exenatide[102,103], semaglutide[104], and dulaglutide[104] not only significantly decreased EAT deposition but also improved glycolipid metabolism disorders. A meta-analysis performed by Berg *et al*[105] confirmed that GLP1-RAs suppressed the abnormal accumulation of EAT. Moreover, liraglutide treatment has been shown to improve LV stiffness and diastolic dysfunction and reduce mortality in HFpEF patients[106]. As a result, GLP1-RAs can inhibit abnormal EAT expansion and may be beneficial for HFpEF. However, further research on this subject is still necessary due to the small numbers of both studies and subjects.

Sodium-glucose cotransporter 2 inhibitors (SGLT2-Is), the newly developed anti-hyperglycemic agents, bind to the SGLT2 transporter in the proximal tubule of the kidney and then promote the urinary excretion of glucose by preventing the reabsorption of glucose[96]. In recent years, SGLT2-Is have been found to play an essential role in mediating anti-inflammatory effects, and therefore its role in regulating EAT has gained significant attention. In individuals undergoing cardiac surgery, Diaz Dí
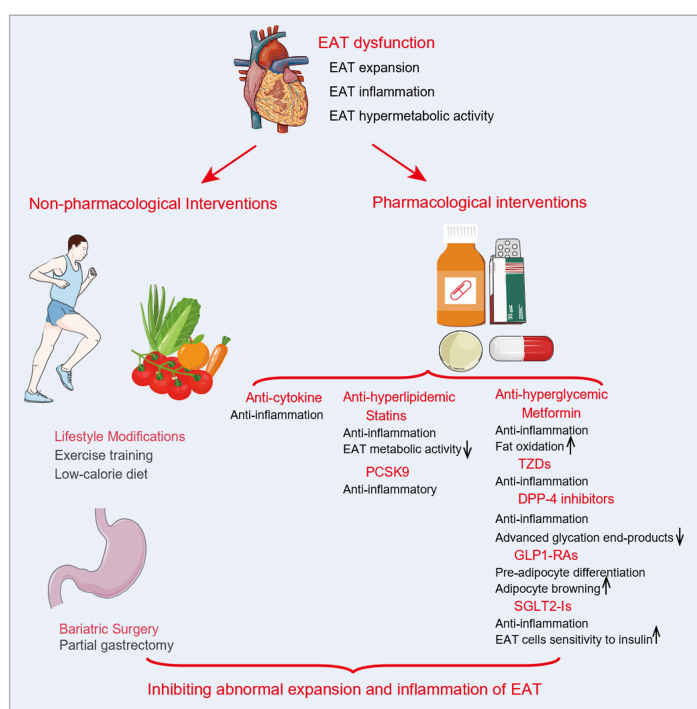
Figure 3 Current interventions targeting epicardial adipose tissue and possible mechanisms. Current interventions targeting epicardial adipose tissue (EAT) reported in the literature include non-pharmacological interventions (lifestyle management and bariatric surgery) and pharmacological interventions related to anti-cytokines, anti-hyperlipidemia, and anti-hyperglycemia. By increasing fat oxidation or sensitivity to insulin and inhibiting inflammation or hypermetabolic activity, these interventions may prevent abnormal expansion and inflammation of EAT. EAT: Epicardial adipose tissue; DPP-4: Dipeptidyl peptidase 4; GLP1-RAs: Glucagon-like peptide-1 receptor agonists; PCSK9: Proprotein convertase subtilisin/kexin type 9; SGLT2-Is: Sodium-glucose cotransporter 2 inhibitors; TZDs: Thiazolidinediones.

az-Rodríguez *et al*[107] demonstrated the expression of SGLT2 in EAT and that dapagliflozin promoted the differentiation of EAT cells and decreased the release of proinflammatory chemokines in *in vitro* assays. Multiple clinical studies have demonstrated that SGLT2-Is (empagliflozin[108,109], dapagliflozin [84,110-112], canagliflozin[113], ipragliflozin[114], luseogliflozin[115]) can dramatically decrease EAT deposition, improve glucolipid metabolism, and reduce inflammatory responses. Conversely, only one study by Gaborit *et al*[116] indicated that empagliflozin failed to reduce EAT volume in patients with T2DM.

A meta-analysis conducted by Masson *et al*[117] confirmed that SGLT2-Is could significantly reduce EAT accumulation and improve glucolipid metabolism. Interestingly, Requena-Ibáñez *et al*[108] reported that empagliflozin could reduce EAT volume in patients with non-diabetic HFrEF. According to Yagi *et al*[113], canagliflozin reduced EAT thickness independent of lowering blood glucose. Thus, SGLT2-Is play an essential role in inhibiting EAT accumulation, possibly independent of glycemic control. Moreover, the current studies confirmed that SGLT2-Is exerts direct pleiotropic effects on the myocardium of HFpEF model animals through multiple mechanisms, such as reducing inflammation, suppressing oxidative stress, and improving cardiac structural and functional dysfunction (myocardial hypertrophy, stiffness fibrosis, and LV diastolic dysfunction)[118-121]. Clinically, SGLT2-Is (empagliflozin and dapagliflozin) have been confirmed to improve exercise tolerance[122] and quality of life in HFpEF patients[123,124] and lower the risk of cardiovascular death or HF hospitalization[125-127]. Consequently, SGLT2-Is exhibit significant prevention of abnormal EAT expansion and positive therapeutic effects in HFpEF, which warrants further clinical validation.

SUMMARY AND FUTURE PERSPECTIVES

T2DM can be one of the essential drivers of the occurrence and development of HFpEF and is associated with a worse prognosis of HFpEF. Systemic inflammation associated with glucose metabolism disorders is a crucial pathological mechanism for HFpEF with T2DM, which is associated with the expansion and dysfunction of EAT. EAT is a facilitator of the pathophysiological process of HFpEF, which may promote inflammation, oxidative stress, myocardial steatosis, and myocardial fibrosis *via* vasocrine or paracrine mechanisms, ultimately contributing to LV remodeling and diastolic dysfunction. Accordingly, inhibition of the expansion of EAT may be an attractive therapeutic intervention for HFpEF with T2DM.

Currently, lifestyle management, bariatric surgery, and certain medications related to anti-cytokines, anti-hyperlipidemia, and anti-hyperglycemia can help to alleviate the inflammation and or accumulation of EAT and reduce clinical symptoms or improve long-term prognosis in patients with HFpEF. Nevertheless, the specific mechanisms by which these drugs inhibit EAT expansion remain to be further explored, and clinical studies on their use in HFpEF with T2DM are lacking. As a result, relevant foundational research and well-designed randomized controlled trials are needed to elucidate the pharmacological mechanisms and efficacy of current interventions. Another critical aspect is to develop new methods to suppress the inflammation or expansion of EAT. Concomitantly, it is essential to thoroughly investigate the mechanisms of abnormal accumulation of EAT so that more novel and effective therapies targeting EAT will become available.

CONCLUSION

In the development of HFpEF with T2DM, the expansion and dysfunction of EAT exert an essential role. Through vasocrine or paracrine pathways, abnormal EAT accumulation may lead to inflammation, oxidative stress, myocardial steatosis, and myocardial fibrosis, resulting in LV remodeling and diastolic dysfunction, which are essential features of HFpEF. Therefore, targeting EAT may be a prospective therapeutic intervention for HFpEF with T2DM. At present, lifestyle management, bariatric surgery, and pharmaceutical interventions may help alleviate the expansion of EAT and improve the clinical manifestations or prognoses of HFpEF patients. Nonetheless, well-designed randomized controlled studies are required to confirm the efficacy of existing treatments. Moreover, it is hoped that more novel and effective therapies targeting EAT will become available in the future.

FOOTNOTES

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Issues and challenges in diabetic neuropathy management: A narrative review

Che Aishah Nazariah Ismail

Che Aishah Nazariah Ismail, Department of Physiology, School of Medical Sciences, University Sains Malaysia Health Campus, Kubang Kerian 16150, Kelantan, Malaysia

Corresponding author: Che Aishah Nazariah Ismail, PhD, Lecturer, Department of Physiology, School of Medical Sciences, University Sains Malaysia Health Campus, Kota Bharu, Kubang Kerian 16150, Kelantan, Malaysia. aishahnazariah@usm.my

Abstract

Diabetic neuropathy (DN) is a devastating disorder with an increasing prevalence globally. This epidemic can pose a critical burden on individuals and communities, subsequently affecting the productivity and economic output of a country. With more people living a sedentary lifestyle, the incidence of DN is escalating worldwide. Many researchers have relentlessly worked on ways to combat this devastating disease. Their efforts have given rise to a number of commercially available therapies that can alleviate the symptoms of DN. Unfortunately, most of these therapies are only partially effective. Worse still, some are associated with unfavorable side effects. This narrative review aims to highlight current issues and challenges in the management of DN, especially from the perspective of molecular mechanisms that lead to its progression, with the hope of providing future direction in the management of DN. To improve the approaches to diabetic management, the suggested resolutions in the literature are also discussed in this review. This review will provide an in-depth understanding of the causative mechanisms of DN, apart from the insights to improve the quality and strategic approaches to DN management.

Key Words: Diabetic neuropathy; Pathophysiology; Diabetic management; Diabetic medication

Core Tip: This review elaborates on the current aspects regarding diabetic neuropathy (DN), especially issues pertaining to the treatments and current challenges in the management of DN with some suggested recommendations on strategies to slow down DN progression. In order to increase the understanding of DN, current lines of therapy, the understanding of its pathophysiology, and future direction of its management are also included. Perhaps, this review may provide insights to understand the important information regarding DN and give ideas for improvement of treatments and management of DN.

INTRODUCTION

Diabetes mellitus (DM), a global public health issue, affects up to half a million people worldwide. According to the World Health Organization (WHO), there was a marked increase in the number of individuals suffering from DM from 108 million in 1980 to as high as 422 million in 2014[1]. In the United States, the Center for Disease Control and Prevention reported that 37.3 million (11.3%) people of the whole United States population are suffering from DM[2]. Diabetic neuropathy (DN) is a common complication of DM that encompasses various patterns of neuropathy as categorised by the location of nerve damage. A recent cross-sectional study among 473 type 2 DM patients from the United Kingdom between 2015 and 2020 demonstrated that the prevalence of diabetic peripheral neuropathy (DPN) was 26.6%, whereby more than half were male patients (52.3%). In terms of DPN severity, 17.3%, 8.2%, and 1.1% of the patients suffered from mild, moderate, and severe DPN, respectively[3]. These statistics showed a huge increase in DN among the DM patient population. Such a worrying trend warrants urgent attention to slowing the DN progression among affected individuals.

Generally, DN can be asymptomatic and only manifests when any disability arises. This disorder affects sensory nerves and it may progress from mild numbness to dysaesthesia, pain, and allodynia eventually. Furthermore, it commonly begins in the feet and lower limbs before spreading proximally [4]. Apart from that, DN may also interrupt motor functions, leading to weakness, atrophy, gait abnormality, and loss of coordination. As a result of the difficulties in performing daily routines, many patients experience a poor quality of life (QOL). DN is also classified as a "length-dependent" neuropathy as it starts at the distal nerve endings of the longest nerve in the lower limbs and extends proximally[5]. In addition, DN can vary in its clinical manifestations. It is categorised either as "painful DN" that manifests as positive symptoms and gain of function (*e.g.*, pain, allodynia, and hyperalgesia) or "painless or insensate DN" that appears as negative symptoms and loss of functions (*e.g.*, numbness and dysaesthesia). Painless DN is a result of the predominant loss of small and large nerve fibres[6] starting at the distal nerve of the limbs before it progresses to the proximal ends in a "glove and stocking" distribution[7]. Despite massive research aimed at identifying the key culprits of DN, its underlying mechanisms remain complicated and unclear[8,9]. Several reviews of DN highlighted the shift in the management towards molecular-oriented approaches. However, the molecular mechanism leading to the progression of DN and its complications remains poorly understood. Consequently, the prevalence of DN continues to escalate and there is a very minimal enhancement in the management of DN. In this review, we aim to highlight current issues and challenges in the management of DN, especially from the perspective of molecular mechanisms that lead to its progression, with the hope of providing the future direction in the management of DN.

PATHOPHYSIOLOGY OF DIABETIC NEUROPATHY

The underlying metabolic abnormalities in DM patients can synergistically drive the development of DN. These abnormalities start with the development of obesity and insulin resistance in type 2 DM (T2DM) or insulin deficiency in T1DM, all of which can result in glucose dysregulation and subsequently, hyperglycaemia and dyslipidaemia[4,10]. In a healthy individual, insulin induces the release of neurotrophic and neuroprotective factors that ensure neuronal survival, as well as C-peptide that restores the structure and function of defective axons. In T1DM patients, as the insulin level falls, the sodium-potassium ATPase (Na^+/K^+-ATPase) and nitric oxide will be disrupted, leading to neuronal dysfunction, oxidative stress, axonal swelling, and apoptosis[5]. Similarly, insulin resistance in T2DM patients may also reduce the anti-oxidant Akt, consequently producing mitochondrial dysfunction, oxidative stress overproduction, and neuronal apoptosis[11]. In addition, the concomitant dyslipidaemia in T2DM patients occurs when free fatty acids are excessively converted by β-oxidation. The

acetyl-CoA transformation during the conversion leads to a great increase in acylcarnitines that are toxic to neurons and Schwann cells[12].

Meanwhile, hyperglycaemia can also activate some pathways that produce excessive polyol, glycation, protein kinase C (PKC), poly (ADP-ribose) polymerase (PARP), and hexosamine, all of which can simultaneously cause overproduction of oxidative stress in the nerves and microvessels[4,5]. In the polyol pathway, the enzyme aldose reductase (AR) converts glucose to sorbitol. This conversion affects a number of downstream reactions that depletes N^+/K^+-ATPase activity, thus reducing nicotinamide adenine dinucleotide phosphate (NADP+) and enhancing the production of reactive oxygen species (ROS), eventually impairing nerve functions[5,6,13,14] and leading to DN. Besides, excessive glucose molecules will enter the hexosamine pathway to produce inflammatory by-products and induce PKC activation secondary to the accumulation of diacylglycerol. Following this activation, insulin resistance is augmented in a way that interrupts the biology of growth factors and causes vasoconstriction of the nerves[10] on top of Na^+/K^+-ATPase dysfunction. As a result, the accumulation of Na^+ leads to axonal swelling and reduced nerve conductivity[5]. Furthermore, the elevated sorbitol and decreased NADH levels trigger ROS increment, glutathione reduction, and cellular osmolarity. Coupled with a decrease in ATP production, these effects can damage mitochondria and DNA as well as reduce the blood supply, eventually speeding up neuronal apoptosis[5]. Additionally, excessive glucose molecules also contribute to the formation of advanced glycation end products (AGEs). When they bind to the receptors (RAGEs), excessive ROS production leads to downstream inflammation that limits blood flow to the peripheral nerves[15]. Although the glycation pathway may take place in the cells of several organs, its effects in DN are more prominent on both myelinated and unmyelinated axons, endothelial cells, pericytes, and Schwann cells[16]. Furthermore, the interference of AGEs on neurofilaments and microtubules of the nerves impedes the axonal transport whilst AGEs formation on the myelin sheath results in localised demyelination[5,16]. Besides, the attack of AGEs on the microvessels increases vascular permeability, hinders vasodilation, stimulates cytokine production, and amplifies oxidative stress levels, all of which lead to a blood flow restriction to the nerves[16]. As more blood capillaries are damaged, the closely connected microvasculature undergoes ischaemia because of the abnormal modification of basement membrane density, pericyte and endothelial cell functions, and arteriovenous shunt formation[5]. All these changes diminish the neuroprotective role of angiogenic factors such as vascular endothelial growth factor. Therefore, the severity of microangiopathy is shown to be associated with impaired nerve conductivity.

The overall pathomechanisms eventually affect the nerves, especially in the peripheral nervous system. Peripheral axons are more fragile compared to motor neurons since they are placed outside the blood-brain barrier. The location also predisposes peripheral axons to injury secondary to DM[10]. Among the different types of peripheral nerves, small unmyelinated C-fibres termed "small fibres" are the most common sensory axons. However, large fibres comprised of small and thinly myelinated Aδ-fibres as well as fully myelinated Aα- and Aβ-fibres are also prone to DN. Patients with DN may experience degeneration and loss of small fibres that result in new-onset pain and prickling or burning sensations (i.e., dysesthesias) in the feet, followed by the initial demyelination or remyelination of the large fibres[12]. Most of the time, the axons that are farthest from the cell body (i.e., located in the feet) are the most severely affected since the number of functional mitochondria produced in their neuronal cell bodies tracking down the axons would be depleted, causing energy deprivation. Amongst the nerve fibres, small fibres are the earliest to be affected due to their structures (i.e., lack of myelination and encapsulation of Schwann cells). Schwann cell encapsulates large fibres to protect axons from external damage and toxic substances. This is an important step in slowing down diabetic-induced progressive energy loss. Therefore, this explains why patients with painful DN often experience pain and dysesthesia as their first symptoms[10]. As diabetes progresses, the myelin sheaths of the nerve fibres undergo degeneration with the detachment of Schwann cells[9]. Subsequently, this leads to even fewer neurotrophic factors being released and eventually, neuronal apoptosis[9]. Consequently, the loss of large axonal fibres causes the patient to experience numbness and loss of proprioception distally in the feet that gradually progress proximally with time. The symptoms usually occur in a symmetrical, distal-to-proximal pattern in all populations of nerves, beginning at the tip of the toes and progressing proximally, giving rise to the "stocking-and-glove" clinical presentation[5,10,17]. Such symptom presentation is regarded as insensate or painless DN whereby the loss of sympathetic regulation of the arteriovenous shunt of the vessels and sweat glands in the foot predisposes the patients to bacterial infections that can later culminate in cellulitis and ulcers[18]. Simplified pathomechanisms of the development of DN are summarised in Figure 1.

Moreover, DN patients are at a high risk of developing diabetic polyradiculopathy, a syndrome that appears together with severe disabling pain in one or more than one distribution of nerve roots and is possibly linked with motor weakness[19]. Besides that, patients with uncontrolled DM and peripheral neuropathy are prone to Charcot neuroarthropathy (CN), also known as Charcot foot, a dreadful condition that can easily originate from microtrauma and neurovascular modifications (i.e., arteriovenous shunting causing the escalation of blood flow and bone resorption)[17,20]. In due time, CN can result in deformities such as collapsed joints and pedal disfigurement[4,17].
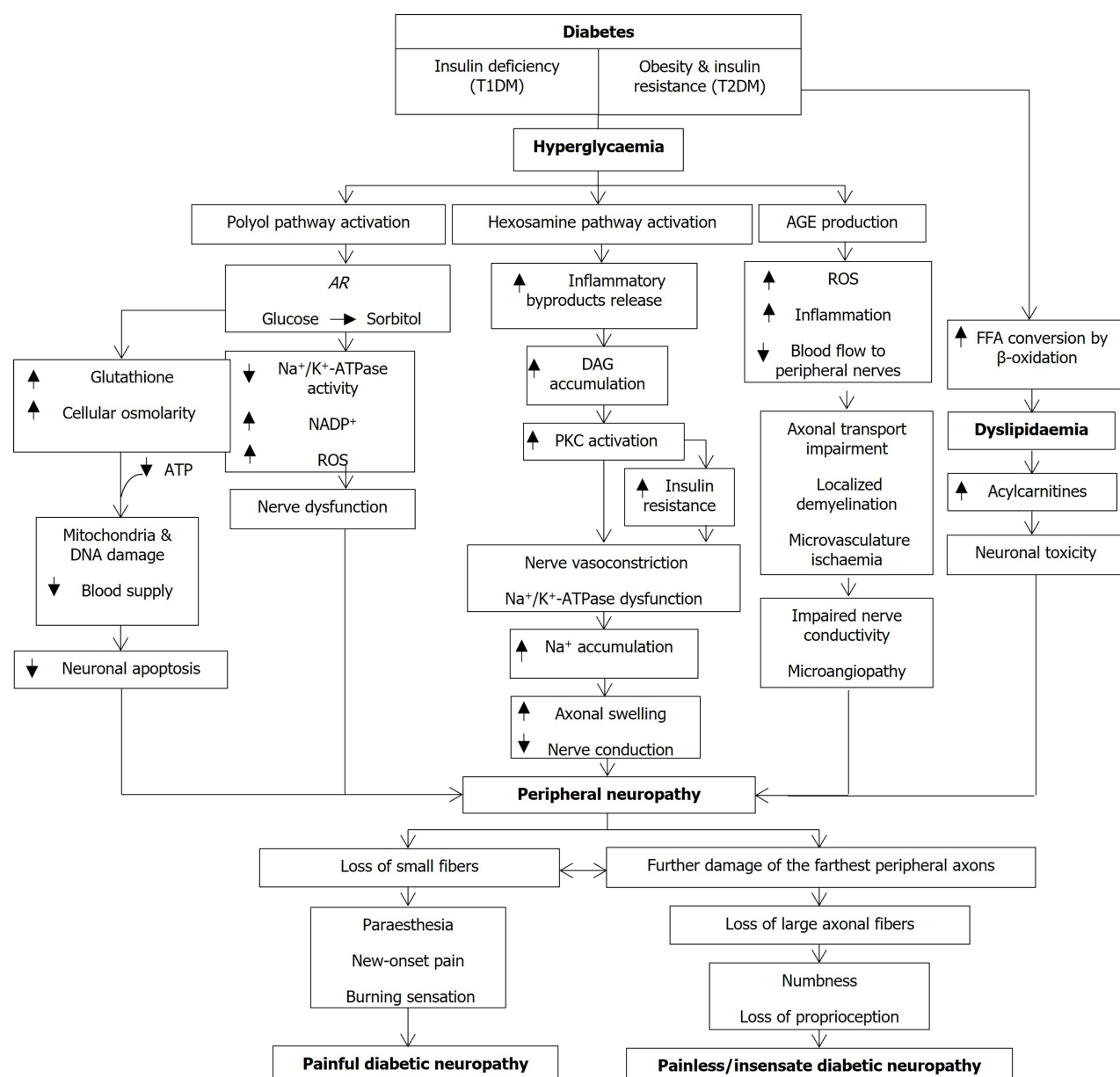
DOI: 10.4239/wjd.v14.i6.741 **Copyright** ©The Author(s) 2023.

Figure 1 Possible pathomechanisms leading to the development of diabetic neuropathy. For further information, see text. PKC: Protein kinase C; T2DM: Type 2 DM diabetes mellitus; ROS: Reactive oxygen species; AGE: Advanced glycation end product; FFA: Food and Drug Administration; NADP: Nicotinamide adenine dinucleotide phosphate.

In view of the wide range of disabling symptoms of DN, various management strategies have been recommended by healthcare professionals to alleviate the symptoms so that the QOL of the affected individuals can be improved.

CURRENT MANAGEMENT OF DIABETIC NEUROPATHY TO DELAY DISEASE PROGRESSION

To date, the management of DN emphasises delaying the progression of neuropathy, reducing the symptoms, and alleviating the complications arising from insensate or painless DN[18,19]. The success of DN management depends on the individual's pathogenic processes[18]. Currently, various clinical guidelines on strategies to prevent and manage DN are available worldwide based on the available published literature. The guidelines encompass a wide range of strategies to prevent the development of DN symptoms, hamper the DN progression, and cure the DN symptoms. Thus, the management of DN can be categorised as preventive or symptomatic approaches[19]. Nevertheless, there are very limited treatment options available for DN that are aimed at underlying nerve impairment. To date, most of the

management strategies emphasise the best way to slow down the progression of DN. Screening for any signs or symptoms of DN is crucial in clinical practice to detect the earliest signs of neuropathy so that prompt intervention can be started[6]. Table 1 outlines the current management to prevent DN progression as elaborated in the literature.

Recommended strategies to delay progression of diabetic neuropathy

First and foremost, DN prevention strategies should begin with blood glucose monitoring and lifestyle modifications[4,6]. Reduction of sweet food can hinder the progression of distal symmetrical polyneuropathy and cardiovascular autonomic neuropathy in patients with T1DM and T2DM[6,21]. However, based on the Diabetes Control and Clinical Trials, this strategy appears to be more effective in T1DM patients whereby their clinical neuropathy is reduced by 60% within 6.5 years following the intensive therapy[22]. In 1998, The United Kingdom Prospective Diabetes Study reported that T2DM patients with neuropathy showed improvement in vibration perception after improvement in blood glucose levels with intensive treatment[23]. However, there was no significant impact of tight glycaemic control on neuropathy among T2DM patients from 1998 to 2015[24]. Although it is suggested that tight glycaemic control could prevent or delay the progression of DN among DM patients, Rodríguez-Gutiérrez et al[24] believed that this strategy alone is inadequate for T2DM patients since they are more likely to suffer from other risk factors such as cardiometabolic factors that are unaddressed[25]. The finding that glycaemic monitoring alone is incapable to slow down the progression of DN in T2DM patients appears to be a new consensus. These patients often suffer from metabolic syndrome that includes obesity, hyperglycaemia, and dyslipidaemia, all of which are critical risk factors for neuropathy[10] as shown in several clinical trials conducted in various countries[24,26-31]. In the United States, The American Diabetes Association (ADA) has implemented different glycaemic target guidelines for children, teenagers, adults, pregnant ladies, and senior citizens in an effort to promote customised care based on individualised glycaemic targets[4,32-35].

Apart from glycaemic monitoring, lifestyle modification is also recommended to reduce cardiometabolic risk factors among T2DM patients to lower the risk of DN and delay its progression. Lifestyle modifications can be in the form of regular exercise and a balanced diet[10]. In animal studies, sustained exercise has been found to: (1) Decrease hyperglycaemia and overproduction of oxidative and nitrosative stress; (2) enhance mitochondrial bioenergetics in the nerve cell body and distal axon; (3) improve microvascular vasoreactivity and reduce nerve ischaemia; (4) elevate axonal transport; (5) counteract the inflammatory effects of dyslipidaemia, lipotoxicity, and obesity; and (6) improve nerve regeneration following metabolic injury[10,36-38]. However, clinical studies involving human subjects reported various outcomes. In 2006, a clinical trial investigating the effect of long-term exercise training on DPN patients reported a significant improvement in peroneal and sural motor nerve functions in the patients[39]. Over the four years of the study period, the development of motor and sensory neuropathy slowed down, thus suggesting that exercise may change the natural course of DN. However, recent studies reported contradicting findings on the effect of exercise on DN. In a randomised controlled trial (RCT) by Stubbs et al[40], 12-wk physical exercise training regardless of type (i.e., sedentary controls, aerobic, isokinetic strength, or a combination of aerobic-isokinetic strength training) did not improve or exacerbate the sensory or motor nerve electrodiagnostic findings (i.e., sural, median, and ulnar sensory nerve responses) in older T2DM patients with length-dependent distal symmetric polyneuropathy. However, a short-term structured program of aerobic exercise was found to selectively improve the sensory nerve functions in a subset of patients. This finding was supported by a recent meta-analysis that included 13 RCTs from 2014 to 2022 with 592 patients that underwent peripheral nerve conduction tests. Exercise, when combined with endurance and sensorimotor training programme, was found to improve balance, glycaemic control, and peripheral nerve conduction, especially in DN patients[41]. Unfortunately, the implementation of such supervised exercise training among the general population in the healthcare system outside of the research setting can be challenging due to patient compliance and shortage of funding, infrastructure, and staff to supervise the patients[4].

In the literature, suggestions have been put forth to include diet observation as part of the prevention strategies in delaying the progression of DN. However, there is a lack of evidence on the effect of diet as the sole prevention strategy for DN since most of the studies incorporated diet as one of the multifactorial lifestyle strategies. For instance, the Diabetes Prevention Program demonstrated that the combination of exercise and diet counselling can reverse the symptoms of metabolic syndrome and lower the incidence of T2DM[42,43]. On a similar note, the ADA also recommends restriction of high-calorie and processed food intake to reduce the risk factors for DN. In turn, the patients should consume food rich in polyunsaturated fats and antioxidants to prevent the development of DN[10]. It is known that lipid metabolites and chronic cellular hyperglycaemia may induce pro-inflammatory cellular injury responses and generate oxidative stress that further diminishes the roles of mitochondria in distal axons [21]. Several dietary supplements are recommended to fight against oxidative damage, including the anti-oxidant α-lipoic acid (ALA). Besides, supplements containing nicotine riboside, a key generator of nicotinamide adenine dinucleotide (NAD+), are also recommended as they can activate certain molecular pathways that shield against dyslipidaemia and obesity[44], resulting in the prevention of oxidative damage in the neurons and delaying the onset of DN[45]. Therefore, dietary management can be effective in alleviating DN. However, it is best to be combined with exercise-based intervention to

Table 1 Management strategies from the previous literature to prevent progression of diabetic neuropathy in patients

Strategies	Description/indication	Intervention/strategies	Ref.
Glucose level monitoring	Prevents distal symmetric polyneuropathy and cardiovascular autonomic neuropathy developments in patients with T1DM, and delays the progression of distal symmetric polyneuropathy in T2DM patients	Treatments (insulin, anti-diabetic medications, electrical stimulation, and percutaneous nerve stimulation; non-treatments (lifestyle modifications such as glucose-dietary control, exercises, and physiotherapy); pancreas transplant; bariatric surgery	[6,4, 132]
Lifestyle modifications	Reduce risk of DN and cardiometabolic causes	Glucose-dietary control; counselling; supervised training programs including physiotherapy/rehabilitation	[4]
Diabetic foot care	Delays or lowers the risk of amputations	Five key elements for prevention of DFUs: (1) Recognition of the at-risk foot; (2) consistent check and examination of the at-risk foot; (3) education of patients, their family, and healthcare providers; (4) routine of wearing suitable footwear; and (5) management of pre-ulceration signs	[47]
Pharmacologic therapeutics	Manage diabetes and neuropathy and treat symptomatic pain	Three suggested phases can be useful: Step 1: Treatment with first-line therapy of TCAs (*e.g.*, amitriptyline), SNRIs (*e.g.*, duloxetine), pregabalin, and gabapentin; step 2: Treatment with second-line therapy including tramadol (weak opioids and SNRIs); step 3: Treatment with last line therapy including strong opioids, cannabinoids, and anticonvulsants Alternatives (anti-oxidant supplementations): α-lipoic acid; acetyl-L-carnitine vitamin B_{12}	[89, 90, 133]

T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; TCAs: Tricyclic antidepressants; SNRIs: Serotonin-norepinephrine reuptake inhibitors; DN: Diabetic neuropathy; DFUs: Diabetic foot ulcers.

ensure a long-term positive impact on glucose and lipid metabolism, as well as axonal regeneration in BM patients[21].

In addition, patients with DN are predisposed to a higher risk of lower extremity amputations. A recent systematic review that evaluated the 5-year mortality rate of patients with non-traumatic below-the-knee amputation and above-the-knee amputation was 40%-82% and 40%-90%, respectively[46], emphasising the importance of annual foot examination and routine foot care in the prevention of lower limb amputations[17]. Education on proper diabetic foot care should be provided to DM patients, including the identification of the at-risk foot, daily examination and inspection, and the use of suitable footgear, as well as accurate and early treatment of pre-ulcerative lesions[47]. The education should also be extended to family members and healthcare providers. Despite the available guidelines on foot care, there is a lack of comprehensive evidence on the best ways to hamper diabetic foot complications. A systematic review of 19 studies demonstrated a reduction in amputation severity, duration of hospital stay, and death rates with proper diabetic foot care. However, the studies were of low quality[48]. In addition, another systematic review of 12 RCTs revealed inadequate high-quality evidence on whether the application of educational strategies alone may minimise the incidence of diabetic foot ulcerations (DFUs) and amputations. The authors agreed that educational interventions should be combined with other interventions in the prevention of DFUs[49].

Current treatments for diabetic neuropathy

Although some non-pharmacological approaches have been introduced to manage the signs and symptoms of DN, anti-diabetic drugs remain the mainstay of DN treatment. Furthermore, there is a paucity of management strategies for individuals with painless or insensate DN as the current therapy focuses on the painful type of DN. Several antidepressants [tricyclic anti-depressants (TCAs), *i.e.*, duloxetine, venlafaxine, and amitriptyline], analgesics (morphine, oxycodone, and tramadol), and anti-convulsants (gabapentin, pregabalin, topiramate, and valproic acid) are prescribed for patients with painful DN. Table 2 summarises the available treatments for DN. Since there is a huge variability in pain between the patients, various types of medications are given to lower painful DN.

Generally, DN will first afflict small nerve fibres such as unmyelinated C-fibres before large fibres (myelinated A fibres), thus explaining the complaints of burning and discomfort among patients with painful DN[18,19]. Pregabalin and gabapentin are the gold standard drugs for pain management[50,51] and are therefore the first- and second-line medications to treat painful DN[4,51]. The exact mechanism of how these anticonvulsants alleviate DN symptoms is unclear. It is postulated that they bind to the α 2δ subunit of calcium channels on presynaptic nerve terminals[52] to induce analgesia. However, these drugs are associated with adverse effects such as tachyphylaxis, somnolence, drowsiness, headache, dizziness, nausea, and diarrhoea[4,51,53]. Furthermore, pregabalin has been linked to misuse and a higher prevalence of deaths, thus there have been calls for its reclassification as a Class C controlled substance in the United Kingdom[54,55].

Apart from that, antagonists of serotonin and norepinephrine reuptake (SNRIs) are also used to reduce DN pain. Similar to pregabalin and gabapentin, duloxetine is recommended as the mainstay of

Table 2 Available therapeutic medications for management of diabetic neuropathy

Management strategy	Therapeutic approach	Description	Contraindications/issues
Pharmacological	Anti-convulsants: Gabapentin; pregabalin	First line medication for painful DN[4,51]; gold standard for pain management[50,51]	Reports on misuse and increased death rate in patients [54]
	SSRI and SNRIs: Duloxetine; venlafaxine	First- and second-line therapy for painful DN[56,57]	Low evidence on venlafaxine effectiveness for painful DN treatment[58]
	TCAs: Amitriptyline; desapramine	First and second-line therapy for painful DN	Associated with constipation, dry mouth, sleep disturbance, sexual dysfunction, somnolence, headaches, arrhythmias, constipation, sleep disturbances, and postural hypotension[4,63]
	Opioids: Tramadol; trapentadol	Opted as acute salvage treatment or as a part of drug combination for painful DN treatment	Strong opioids are frequently associated with therapeutic abuse and misuse[68]; use of tramadol is more preferred due to reduced risk of abuse or misuse[68]
	Sympathetic blocking agents (α-adrenergic antagonists): Clonidine; regitine; phenoxy-benzamine	One of the opted therapies for complex regional pain syndrome treatment[72]	Limited evidence in RCT testing the drug's efficacy in painful DN patients; efficiency of clonidine depends on relative functionality of nociceptors in painful DN patients, however no statistical significance is achieved although the trends of efficacy is shown[70]
Non-pharmaco-logical	Sympathetic nerves blockade: Lumbar sympathetic nerves blockade; combined strategies of lumbar sympathetic pulsed radiofrequency and continuous epidural infusion; combined treatment of continuous sympathetic block and neurolysis with alcohol	Recommended for severe painful DN patients who failed to any pharmacological treatments	Patients demonstrated improved life expectancy, greater DN symptom improvement, satisfactory safety, rapid recovery, and rapid relief of pain[73-76]; associated with several limitations of additional diagnostic tools, small size population, short period of follow-up, and issue regarding combined treatment duration[75,76]
	Capsaicin	Recommended for patients with intolerable oral therapeutic consumption[4]	Low to moderate level of evidence for topical capsaicin efficacy[82,83]; associated with small nerve fibers injury and disturbed nociceptive signaling[84]
	Neuromodulation devices: FREMS; SCS, NMES; TENS	Studies on their efficacy in painful DN is still on-going	Not yet approved for clinical guidelines for painful DN treatment due to very low evidence of efficacy[4,85,86]
	Nutraceuticals: ALA; ALC; vitamin B_{12}	ALA improves numbness and paraesthesia with reduced side effects[89]; vitamin B_{12} is recommended to T2DM patients with metformin prescription[90]	There is a lack of standardization in quality and manufacturing of nutraceuticals[91,92]; low safety level due to less evidence of high-quality studies[87,93]

TCAs: Tricyclic antidepressants; FREMS: Frequency-modulated electromagnetic neural stimulation; SCS: Spinal cord stimulation; NMES: Neuromuscular electrical stimulation; TENS: Transcutaneous electrical nerve stimulation; ALA: α-lipoic acid; ALC: Acetyl-L-carnitine; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.

treatment for painful DN. It attenuates the descending pain mechanisms and moderately hinders dopamine reuptake. Apart from producing similar side effects as anticonvulsants, this drug also unfavourably affects sexual functions and sleep[6]. Another selective serotonin reuptake inhibitor, venlafaxine, is also recommended by the European Federation of Neurological Societies Task Force and the American Academy of Neurology as a therapy for painful DN[56,57]. However, based on a previous Cochrane systematic review of six RCTs and 460 participants comparing the placebo effect with a venlafaxine dosage of 150-225 mg, the level of evidence for its effectiveness is low[58].

Apart from that, tricyclic antidepressants such as amitriptyline are also recommended for painful DN [4], especially acute pain[59]. Several RCTs have reported its effectiveness in alleviating painful DN[60]. In a RCT, Kaur et al[61] compared the efficiency of duloxetine and amitriptyline. They found a similar efficacy of these drugs in treating patients with painful DN. The mechanism of TCAs in targeting painful DN is not understood, but amitriptyline is found to attenuate the reuptake of serotonin and noradrenaline at the nerve terminals and ion channels (sodium and potassium ion channels), as well as N-methyl-D-aspartate receptors (NMDARs) in the central nervous system[62]. However, amitriptyline is associated with side effects such as constipation, dry mouth, sleep disturbance, sexual dysfunction, somnolence, headaches, arrhythmias, sleep disturbances, and postural hypotension[63]. Apart from amitriptyline, other TCAs such as desipramine and nortriptyline have also been investigated as potential treatments for painful DN. Several RCTs reported a reduction in painful DN symptoms following desipramine treatment[64-66], making it likely to be as effective as amitriptyline[64] with lesser side effects[65].

On top of that, some clinical guidelines recommended opioids be included as one of the treatments with or without other drugs for DN patients with severe pain intensity[67]. However, opioid is frequently associated with therapeutic abuse and misuse. Tramadol, one of the opioids, is fairly acceptable in the treatment of moderate to severe pain as it has a lower risk of abuse or misuse. It reduces pain by binding to opioid receptors (*i.e.*, κ-, δ-, and μ-receptors) centrally besides mitigating the serotonin and norepinephrine reuptake, thus augmenting the inhibitory effects of pain transmission in the spinal cord dorsal horn[68]. Apart from that, tapentadol is also suggested for the treatment of painful DN in the US. It shares a similar mechanism of action with tramadol, except for a higher affinity for μ-receptors. However, the level of evidence to show the efficacy of these opioids was low based on the above-mentioned Cochrane systematic review that included six RCTs and 438 participants[69].

Pertaining to the potential to target the C-fibres in peripheral sympathetic nerves, the use of sympathetic blocking medications (α-adrenergic antagonists) such as clonidine, regitine, or phenoxyben-zamine is recommended in some studies to improve the pain secondary to the spontaneous firing of the affected nerve fibres[18,19]. An RCT conducted by Campbell *et al*[70] demonstrated that the level of foot pain subsided after the topical application of clonidine gel among patients with painful DN. However, the effectiveness of this medication relies on the relative functionality level of nociceptors (*i.e.*, functional and possibly sensitised nociceptors in the affected skin). This trial failed to achieve significant results despite showing some evidence of the drug's efficacy. Another earlier RCT on transdermal clonidine application in diabetic polyneuropathy patients also failed to achieve promising results as drug withdrawal effects and pain recurrence were reported among the trial participants[71]. Even though this sympathetic blocking agent can be used to treat other complex regional pain syndromes, there is still very scarce analysis with regard to painful RN in the Cochrane database. This was concurred by Mackey *et al*[72] who reported that not only this class of medication did not show any efficacy in treating neuropathic pain, its use was challenging due to the side effects profile.

In some cases of patients with persistent severe painful DN despite multiple pharmacological approaches, shifting the pain relief mechanism to the sympathetic nervous system can possibly assist the management of the severe pain. In a clinical trial, permanent lumbar epidural blockade was found to produce satisfactory outcomes when several other pharmacotherapeutics failed to treat patients with painful DN[73]. In another case reported by Cheng *et al*[74], a painful DN patient who was unresponsive to several medications showed significant pain relief following the blockade of nine lumbar sympathetic nerves over a 26-mo duration. His QOL was further improved over the two years. Further advancement of this approach, *i.e.*, lumbar sympathetic pulsed radiofrequency combined with continuous epidural infusion, appeared to successfully manage painful symptoms of DN in the patients [75]. Meanwhile, the combined treatment of continuous lumbar sympathetic block and neurolysis with alcohol also produced a greater improvement of DN symptoms and rapid recovery in the patients, not to mention its satisfactory safety profile[76]. However, there are certain limitations to this approach, such as the requirement for additional tools to assess and diagnose the severity and duration of DN. Furthermore, the small size population, short period of follow-up, and duration of the combined treatment strategies in the previous studies[75,76] restrict the generalisability of the results, thus further research is warranted.

Additionally, unmyelinated C-fibres release neurotransmitter substance P during the transmission of pain signals from the periphery to higher centres. This pathway could be blocked by the topical application of capsaicin[77], especially for patients with localised pain who are unable to tolerate oral medications[4]. Previous reports have demonstrated its effectiveness in improving nerve functions and lowering pain sensations in painful DN patients at a dosage of 0.075% four times a day[78,79]. Meanwhile, DN can also affect myelinated A-fibres that produce deep-seated, dull, and distressing pain that is usually unresponsive to sympathetic blocking agents and capsaicin[18,19]. This natural product blocks pain transmission by modifying the membrane potential of vanilloid receptor subtype 1 and certain ion channels, as well as the neurotrophic signalling at the nerve fibres[19,80]. Besides, it can also initiate acute production of vasoactive peptides from perivascular sensory terminals following topical application[81]. The use of topical capsaicin to treat painful DN is approved by the Food and Drug Administration and the level of evidence for its efficacy ranges from moderate to low[82,83]. On the downside, several reports have emerged regarding the potential side effects of topical capsaicin in damaging small nerve fibre and interrupting nociceptive signalling[84].

Besides the above-mentioned pharmacological strategies, there are other alternative approaches to alleviate the symptoms of painful DN. Neuromodulation strategies using specific devices such as frequency-modulated electromagnetic neural stimulation (FREMS), spinal cord stimulation (SCS), neuromuscular electrical stimulation (NMES), and transcutaneous electrical nerve stimulation (TENS) represent new hopes for DN patients[4]. However, these strategies are still under investigation and not included in any clinical guidelines to treat DN as the level of evidence is very low[4,85,86]. Similarly, alternative complementary approaches such as acupuncture and static magnetic field therapy have also been used to manage painful DN[19]. Nevertheless, data on these management strategies are also limited.

Furthermore, a series of clinical trials have demonstrated the efficacy of the antioxidant nutritional supplement, *i.e.*, ALA, acetyl-L-carnitine, and vitamin B_{12} in alleviating the pain linked to DN[57,87,88]. An oral supplement of ALA at 600 mg per day may reduce DN pain within 2 wk, besides improving

numbness and paraesthesia symptoms with minimal adverse effects[89]. Similarly, ALA lowers pain intensity by decreasing oxidative stress that afflicts nerves and microvessels after metabolic modifications[4]. Meanwhile, the regular supplementation of vitamin B_{12} is recommended especially for T2DM patients who are on metformin to offset the side effect of vitamin B_{12} deficiency[90]. Despite promising outcomes, worldwide availability, affordable cost, and being regarded as a "safer option", there are concerns regarding these nutraceuticals in terms of lack of regulations including standardisation in manufacturing and quality control[91,92]. Furthermore, the safety profile of these nutraceuticals remains unclear due to the lack of high-quality clinical trials[87,93].

ISSUES AND CHALLENGES IN DIABETIC NEUROPATHY MANAGEMENT

Since the prevalence of DN is rapidly rising, multiple strategies in terms of treatments, new therapeutic approaches, patient access to healthcare facilities, and provision of knowledge regarding DN have been introduced to slow down the disease progression. Unfortunately, several ongoing issues must be resolved in the management of DN. This section elaborates on the issues and challenges in improving the management of DN from the aspect of treatment, patient adherence, access to facilities, and knowledge.

Issues in diabetic neuropathy treatments

In the literature, a number of observational and interventional studies revealed that half of the patients with DM develop the signs and symptoms of DN during their lifetime[6,87,94-96]. The prevalence of DN is high (approximately 20%-30% in newly diagnosed and early-stage T2DM)[30]. Additionally, it is challenging to treat DN patients with symptomatic (painful) variants since the pain can be debilitating and excruciating. They often complain about pain sensation over the lower extremities that is apparent at rest and intensifies during night time[19]. Unfortunately, the exact pathogenesis of this illness is unknown. Many clinical trials failed despite promising outcomes in pre-clinical studies. Therefore, novel disease-modifying medications are scarcely developed because of the doubts surrounding pharmacological targets.

On a further note, since the role of aldose reductase in the pathogenesis of DN was discovered by Dvornik *et al*[97], it has been extensively investigated due to its promising effects in reversing DN. Combating DN by antagonising this enzyme seems to be a promising step[14]. The application of aldose reductase inhibitors (ARIs) has been shown to hamper the overactivity of the polyol pathway. However, a previously published systematic review did not pinpoint a single RCT showing any superiority in ARIs compared to placebo in DN patients[98]. Although it has been three decades since the first discovery of ARIs, these drugs are still not established as the mainstay of DN treatment due to a high incidence of side effects[99]. Similar issues were also raised for other potential therapeutics involving the antagonism of PKC activation resulting from excessive diacylglycerol accumulation. A systematic review of RCTs on the application of the PKC inhibitor ruboxistaurin (RBX) has reported its therapeutic effects on DN. However, the evidence from those studies was insufficient to establish its efficiency in treating DN[100]. Moreover, RBX has been shown to be more effective in relieving symptoms among patients with less severe DN[100,101].

Last but not least, other potential new drugs targeting RAGEs activation have also been extensively explored in animal models[102,103], some of which have produced encouraging therapeutic effects in patients[104]. However, the high toxic contents of these drugs become a major problem in human trials [10,15]. Due to these uncertainties and suboptimal therapeutic efficiency in improving nerve functions in T2DM-induced DN[15], the industry refuses to invest further in such drugs[5]. Thus, it limits the available medication option for patients. They have to rely on the combination of anti-diabetic medications with other management strategies to delay the progression of DN.

Challenges in patients' adherence to diabetic neuropathy medications

Although diabetic management guidelines have been established worldwide, not all patients can adhere to the recommended strategies due to many factors. Patients' non-adherence to T2DM treatment regimens continues to be a major issue in most countries[105,106]. It is closely related to poor knowledge regarding diabetes aetiology and disease progression, unstable socioeconomic status, poor family support, patient-staff engagement barriers, complex therapeutic regimens, and lack of medical insurance coverage[105-108]. Some patients even voluntarily stopped the treatment plan and shifted to traditional herbs following their concerns about the side effects of the medications.

Moreover, unsatisfactory healthcare also contributes to the non-adherence to self-care diabetic management[106]. Even with free medications provided by the government, patient adherence can be compromised if there is ineffective communication between the patients and healthcare providers[106]. It is undeniable that myths and cultural beliefs would influence the faith of a patient in doctors' prescriptions and recommendations, especially if the patient lacks an understanding of disease progression[105,109]. Therefore, it is vital to provide appropriate health education and counselling to increase the patient's adherence rate. As proven by Awodele and Osuolale[110], patients' clinical

outcomes improved significantly (*i.e.*, 86.8% adherence rate) following health education and counselling.

Besides that, a complex treatment regimen can also contribute to non-adherence. Patients with multiple comorbidities generally have more medications from different pharmacological classes, giving rise to polypharmacy. A cross-sectional study among diabetic patients with no comorbidities demonstrated a higher adherence to diabetic medications[111] as compared to patients with comorbidities who required multiple medications[112,113]. This is further complicated by the poor awareness of the importance of diabetic medications, especially in rural areas of low-income countries [105,114,115]. However, this issue can be addressed by involving the community and healthcare providers to improve the awareness of the patients. Evidently, encouragement from family and friends has been linked with an improvement in patients' knowledge and adherence to dietary recommendations[106]. Moreover, elderly patients with multiple comorbidities displayed better medication adherence when provided with more information on the benefits[116,117].

Poverty leads to poor management in DN

Although comprehensive diabetic management has been established and practised globally, not all are fully attainable, especially in low-income or developing countries with high rates of poverty. Financial restraint often leads to the non-adherence of patients. In Nigeria, 51% of diabetic patients, most of who were women and unemployed, could not afford DM medications. Another 69% had to purchase their medications in smaller dosages due to high costs[110]. To minimise these obstacles, support from high-income countries is crucial. National programmes in medical schools, health centres, and hospitals can be put in place under international collaborative partnerships[118]. Evidently, a 12-mo Kerala Diabetes Prevention Programme made up of a peer support education group led to significantly improved lifestyle changes and lower cardiovascular factors among the participants. However, there was an insignificant outcome for diabetic symptom improvement[119].

Restricted access to facilities and patient education due to the coronavirus disease 2019 pandemic

It is undeniable that the coronavirus disease 2019 (COVID-19) pandemic has cast a huge impact on the healthcare and management of many diseases, including DM. During the pandemic, a prolonged lockdown was implemented. In many low-income countries, there was a lack of proper guidelines for DM patients to attend follow-ups in hospitals. Furthermore, with the low coverage of sick pay or social security, people from low-income countries were less likely to practise preventive measures such as social distancing, the use of protective gear, and visiting emergency health services. Furthermore, since diabetic management requires a visit to healthcare centres for drug prescription, many patients faced restricted access to medications. Insulin was especially restricted during the COVID-19 outbreak. At some point, many outpatient clinics and endocrinologists at private hospitals were temporarily shut down while the focus of emergency services shifted to the treatment of COVID-19 patients. These difficulties affected the care of diabetic patients, especially those who required hospital admission[120]. In short, the interruption of routine diabetic care created stress among patients, not to mention worsening obesity due to physical inactivity, both of which worsened their hyperglycaemic conditions and diabetes-related complications[121].

As the crisis of COVID-19 unfolds over the past two years, new strategies were developed to enhance diabetes care, including the use of telehealth, remote patient monitoring, online glucose monitoring *via* wearable technologies supported by the internet and smartphones, and free educational videos and e-books on self-management of diabetes *via* mobile applications[122-124]. However, these guidelines are established in developed countries, making them less suitable for patients in low-income countries with issues like poverty, poor education level, and suboptimal healthcare planning. Several suggestions were put forth to potentially improve the care of DM patients, such as replacing active follow-up with passive care, establishing community centres for patient visit and training purposes outside the hospitals (*e.g.*, in mosques, churches, and community centres), and setting up more outpatient clinics and primary healthcare centres for the treatment of non-communicable diseases. At these centres, innovative steps were proposed and implemented, including self-monitoring of blood glucose levels without additional charges, guidelines for physicians on clinical management cases during disease outbreaks, needs assessment survey by trained investigators, and contacting patients *via* landlines for consultation with physicians and endocrinologists, as well as spreading educational and intervention information *via* text messages for patients with smartphones[120].

FUTURE DIRECTION IN MANAGEMENT OF DIABETIC NEUROPATHY

It is crucial to implement strategies to prevent and slow the progression of DN, especially since severe DN can be challenging to treat. There are many suggestions to achieve this. In T1DM patients, not only are the insulin-producing pancreatic β-cells destroyed, but the blood capillaries are also hugely affected. Blood capillaries are critical in insulin production; thus, it is vital to manage capillary destruction. A new drug from bone marrow stem cells has been developed to replenish the cells of blood capillaries

and increase the production of β-cells. This intervention is based on the concept of introducing the formed β-cells in the form of "immunoprotective capsules" to avoid destruction by auto-immune cells [125]. This research is still ongoing. Issues related to the capability of multipotent stem cells in the formation of β-cells that can potentially proliferate into cancerous cells need to be fully addressed before the application of this drug[126]. Besides that, other proposed methods include dietary changes in DM patients, such as the consumption of amino acid arginine to facilitate the metabolism of glucose as has been proven in animal studies[125]. Arginine stimulates the production of glucagon-like peptide-1 from endocrine cells in the gut following nutrient ingestion that can promote insulin secretion, reduce food intake, increase β-cell production, and minimise β-cell apoptosis[127].

Lastly, there is growing research in the area of metabolomics technology that may aid in the diagnosis and biomarker discovery of DM. Since metabolites reflect the whole body's functions, it is hypothesised that they can provide a comprehensive picture of what happens in the body. The combination of metabolomics detection technology with computational biology and orthogonal experiments allows the screening of diabetic metabolites and evaluation of the related metabolic pathways[128]. Evidently, through metabolomics research, it is discovered that T1DM children who developed auto-antibodies before the age of 2 had twice the depletion rate of methionine level compared to the children who developed autoantibodies in later childhood or children who were auto-antibody-negative. The same research also speculated that the methionine pathway could be involved in the generation of antibodies during early infancy[129]. Following that, a metabolomics study using transgenic and knock-out mouse models that resembled early stages of human T1DM also revealed metabolomics disturbances before the onset of T1DM. In their study, Overgaard et al[130] found a reduced level of lysophosphatidylcholine and methionine as compared to an elevated level of ceramides before the onset of T1DM. Meanwhile, in a study on insulin autoantibody seroconversion among diabetic children, Li et al[131] discovered that the rapid growth of children's height is linked to an increased risk of islet autoimmunity and progression of T1DM. These published studies represent the growing metabolomics research that has made great progress in the identification of the main factors and metabolites that helps to identify the pathophysiological process, aetiology, early prevention, and assessment of the treatment effects of diabetes.

DISCUSSION

Primary resources of diabetic care from published studies serve as the general guidelines for better diabetic prevention strategies and patient care worldwide. Along with lifestyle and dietary modifications, additional strategies need to be added to the guidelines for the betterment of diabetes care. For instance, glucose monitoring is one of the current strategies that has been proven effective in controlling blood and dietary glucose in the previous literature for T1DM patients. However, this strategy is more beneficial in reducing diabetic complications and progression for T1DM patients because the pathogenesis of DN differs between T1DM and T2DM. For example, hyperglycaemia is not the key factor to all the complications suffered by T2DM patients. In view of this, the general management of DN among T1DM and T2DM should be tailored accordingly. It is also important to note that over-aggressive glucose control can lead to hypoglycaemia-induced neuropathy in T2DM patients. It is especially devastating for neurons in the brain that use more glucose than other cells to fulfill their functions.

There are certain misconceptions regarding the dietary monitoring of glucose intake among DM patients. Most diabetic patients eliminated sugars in their beverages but fail to reduce the consumption of carbohydrate-rich meals and sugar-rich fruits, especially in countries where carbohydrate-rich food and exotic fruits are the staple diets. The consumption of these foods may complicate the diabetic condition and accelerate the progression of DN. Therefore, it is critical to disseminate accurate knowledge about dietary glucose through social media to avoid any misconceptions among diabetic patients.

Besides that, poor treatment adherence is also a major challenge in the prevention and management of DN as discussed in the previous section. In countries with traditional lifestyles such as Asian countries, many patients opted for herbal medicine rather than modern medications, possibly due to concern about side effects and a lack of trust towards modern medicine. Although some of the traditional herbs demonstrate a potent anti-diabetic effect, the herb preparation by local manufacturers may contain additional harmful substances such as steroids that can lead to other complications. Furthermore, the crude extracts of certain herbs can be unsafe as some of the unknown metabolites can worsen the diabetic condition. Therefore, governmental agencies should conduct strict screening of the content of traditional anti-diabetic herbs before it is commercialised to reduce the risk of complications. More importantly, patient education and continuous research on these new anti-diabetic agents should be emphasised by the government as a step to improve diabetic management.

CONCLUSION

The increasing prevalence of DN and its complications among DM patients is alarming and can be costly to individuals and countries alike. Recently, psychosocial impact and morbidity from DN have also received widespread concern. Current clinical guidelines focus on preventing the progression of DN and managing the DN symptoms in patients. However, most of these guidelines fail to address the underlying factors contributing to DN, thus compromising the effectiveness of current management. Therefore, it is crucial to identify the mechanisms and risk factors of DN so that issues hindering the success of the current management of DN can be resolved. This review outlines various challenges in the management of DN on top of the pathomechanisms of DN. With a better understanding of DN pathogenesis, DN management can be enhanced. It is hoped that the additional recommendations pertaining to the raised issues can be addressed for the betterment of the quality of care and patients' health.

FOOTNOTES

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Adiponectin as a therapeutic target for diabetic foot ulcer

Mona Mohamed Ibrahim Abdalla, Jaiprakash Mohanraj, Sushela Devi Somanath

Mona Mohamed Ibrahim Abdalla, Department of Physiology, Human Biology Division, School of Medicine, International Medical University, Kuala Lumpur 57000, Malaysia

Jaiprakash Mohanraj, Department of Biochemistry, Human Biology Division, School of Medicine, International Medical University, Kuala Lumpur 57000, Malaysia

Sushela Devi Somanath, Department of Microbiology, School of Medicine, International Medical University, Kuala Lumpur 57000, Malaysia

Corresponding author: Mona Mohamed Ibrahim Abdalla, MSc, PhD, Senior Lecturer, Department of Human Biology, School of Medicine, International Medical University, No. 126 Jln Jalil Perkasa 19, Bukit Jalil, Kuala Lumpur 57000, Malaysia. monamohamed@imu.edu.my

Abstract

The global burden of diabetic foot ulcers (DFUs) is a significant public health concern, affecting millions of people worldwide. These wounds cause considerable suffering and have a high economic cost. Therefore, there is a need for effective strategies to prevent and treat DFUs. One promising therapeutic approach is the use of adiponectin, a hormone primarily produced and secreted by adipose tissue. Adiponectin has demonstrated anti-inflammatory and anti-atherogenic properties, and researchers have suggested its potential therapeutic applications in the treatment of DFUs. Studies have indicated that adiponectin can inhibit the production of pro-inflammatory cytokines, increase the production of vascular endothelial growth factor, a key mediator of angiogenesis, and inhibit the activation of the intrinsic apoptotic pathway. Additionally, adiponectin has been found to possess antioxidant properties and impact glucose metabolism, the immune system, extracellular matrix remodeling, and nerve function. The objective of this review is to summarize the current state of research on the potential role of adiponectin in the treatment of DFUs and to identify areas where further research is needed in order to fully understand the effects of adiponectin on DFUs and to establish its safety and efficacy as a treatment for DFUs in the clinical setting. This will provide a deeper understanding of the underlying mechanisms of DFUs that can aid in the development of new and more effective treatment strategies.

Key Words: Diabetic foot ulcer; Adiponectin; Anti-inflammatory; Adipose tissue; Antioxidants; Wound healing

Core Tip: The global burden of diabetic foot ulcers (DFUs) is significant, both in terms of human suffering and healthcare costs. Therefore, effective strategies to prevent and treat DFUs are urgently needed. Adiponectin, a hormone produced by adipose tissue, shows promise as a therapeutic option for DFUs due to its anti-inflammatory, antioxidant, and pro-angiogenic effects. While adiponectin has potential therapeutic applications, further research is necessary to establish its safety and efficacy in clinical settings. This review aims to summarize current research on adiponectin's potential role in treating DFUs and identify areas requiring further investigation.

INTRODUCTION

Diabetic foot ulcers (DFUs), a serious complication of diabetes mellitus, pose a significant economic burden on patients and healthcare systems worldwide. The development of these ulcers is often due to poor foot care, inadequate glycaemic control, underlying neuropathy, and peripheral vascular disease. Left untreated, these ulcers can result in amputations. The global prevalence of DFUs ranges from 3% in Oceania to 13% in North America, with a global average of 6.4%[1].

Healing time for these ulcers can take up to 12 mo, with a recurrence rate estimated to be 65% within 5 years[2]. Studies have shown that the impact of DFUs on individuals is profound, with loss of ambulatory function, financial strain, and emotional suffering being common outcomes[3]. The economic impact on patients and their families due to medical bills, loss of income, and emotional distress can be significant. Participants in a recent study reported experiencing depression, isolation, and hurtful comments from others[3].

DFUs continue to pose a significant public health challenge, and they are a major cause of morbidity and mortality worldwide[4].

Adiponectin, a fat-derived hormone, has been shown to protect against insulin resistance, type 2 diabetes (T2DM), and atherosclerosis. Reduced circulating levels of adiponectin are thought to play a role in the development of T2DM. In cases of obesity, the production of endogenous adiponectin is impaired. It is, therefore, suggested that pharmacological or dietary interventions be considered to restore the capacity of adipose tissue to secrete adiponectin[5].

DIABETIC FOOT SYNDROME

Diabetes mellitus (DM), is a main cause of death and poor quality of life worldwide, affecting 463 million individuals in 2019 and is estimated to reach 700 million by 2045[2]. People with diabetes often have foot problems that impose an economic burden on the individual, and about half of all foot amputations are observed to be among diabetics. The lifetime chance of a diabetic having a foot ulcer is as high as 25%[3], and it is estimated that every 30 s a lower limb is lost due to diabetes somewhere in the globe[4]. DFUs can be prevented by ensuring that diabetics get regular foot exams and treating any neuropathy that may be present[5]. The International Diabetes Foundation has called for greater awareness of diabetes foot concerns due to the psychological, social, medical, and economic effects of what should be one of the most preventable long-term complications of diabetes[6,7]. In most Western nations, the yearly incidence of DFUs is roughly 2%, however, greater rates have been observed in select populations, including Medicare recipients (6%) and United States veterans (5%)[8]. The projected annual cost to the NHS in the United Kingdom is around 580 million, with 307 million spent on ulceration in primary care[9].

Diabetic foot syndrome is defined, according to the World Health Organization, as "ulceration of the foot (distally from the ankle and including the ankle) associated with neuropathy and different grades of ischemia and infection"[10]. It is a significant, long-term consequence of diabetes that can result in amputations, disability, and diminished quality of life.

Since peripheral neuropathy and vascular disease are present in more than 10% of individuals at the time of diagnosis of T2DM[11] and because the first year following the diagnosis of diabetes is a risky time for foot ulcers and amputations, the burden of diabetic foot disease is expected to rise in the future [12]. Furthermore, emerging nations in Africa, Asia, and South America, where foot ulcers are more likely to have neuropathic origins[13] and are thus very avoidable, are anticipated to have the biggest growth in the prevalence of T2DM[6]. Deploying screening, education, and treatment programmes most effectively around the globe is still the dilemma facing the worldwide diabetes community[14].

The simplest definition of diabetic foot infection is "any infra-malleolar infection in a diabetic patient". These include paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis, and osteomyelitis. DFUs are complex and rarely caused by a single condition. Several risk factors cause DFUs[15,16]. Understanding pathobiology helps diagnose and treat DFUs, which is one of the leading indicators for amputations.

Neuropathy is the primary contributing factor leading to ulceration, in diabetics. Diabetic peripheral neuropathy (DPN) is a disruption of normal nerve function that can change autonomic, motor, and sensory functioning throughout the body[17]. Due to the absence of protective sensation in patients with sensory neuropathy, the foot is more likely to sustain untreated minor injuries as a result of excessive pressure as well as mechanical or thermal damage. Individuals with diabetes who also have sensory neuropathy were found to be at the highest risk for developing ulcers, as revealed by a significant prospective multicentred investigation[18].

There are various types of neuropathies, and some of them may cause foot ulcers. Motor neuropathy may lead to foot deformities, decreased joint mobility, and abnormal foot loading. These modifications may cause a shift in the distribution of loads that are experienced when walking, with a subsequent reactive thickening of the skin known as callus at unusual load areas. Ischaemic necrosis of the tissues underneath the callus also contributes to the development of a neuropathic ulcer. Autonomic neuropathy often results in changes to the skin's texture and turgor, such as dryness and fissuring, which makes the skin more susceptible to infection since it provides an entry site to the bacteria[19].

Another condition that contributes to the development of foot ulcers is peripheral vascular disease, which affects both small and major blood vessels. It is possible for both macrovascular and micro-vascular diseases to contribute to the symptoms of peripheral vascular disease, which ultimately results in a delay in wound healing. In both diabetics and non-diabetics, there is an increase in the incidence and prevalence of peripheral arterial disease with age, while the condition is worse with diabetes. Individuals who have diabetes are at an increased risk for vascular disease because of the prevalence of risk factors such as hypertension, smoking, and hyperlipidaemia[20,21].

The ulcerated diabetic foot is the result of a complex interaction between several factors, including neuropathy, peripheral vascular disease, trauma, and infections. Neuropathy and ischaemia, also called neuro ischaemia, are the initial mechanisms, while the infection is typically a result of this condition. Studies have indicated that diabetics acquire peripheral vascular disease at a younger age more frequently than others in the same age group[22]. In 35% of cases, proximal arterial disease-related peripheral ischaemia was cited as an important cause of ulceration among diabetics in a two-center study of causal pathways[22]. In another study that compared diabetic patients with peripheral artery disease to non-diabetic patients with the same condition, it was found that diabetic patients had more distal disease and a worse prognosis in terms of amputation and mortality[23].

Hence the pathogenesis of DFUs, a complication of longstanding uncontrolled diabetes, involves multifactorial influences such as neuropathy, peripheral vascular disease, foot deformity, trauma, infection, and inadequate glycaemic control. The loss of sensation brought on by neuropathy can result in repeated damage to the foot, while the peripheral vascular disease can reduce blood flow and slow healing. Injuries and infections can exacerbate already-existing ulcers, while foot abnormalities can create pressure points and raise the risk of skin deterioration. Moreover, poor glycaemic management might hinder wound healing and raise the danger of infection. Thus, for the prevention and management of DFUs, a multidisciplinary strategy that takes these aspects into account is essential. Figure 1 summarizes the factors contributing to the development of DFUs in diabetic patients.

RISK FACTORS

Multiple variables contribute to the emergence of DFUs. Peripheral neuropathy and ischaemia that result from the peripheral vascular disease that reduces the protective components of the tissues are the primary underlying causes. In addition, the skin can be subjected to stress, such as pressure, shear, or trauma, which also contributes to the condition. Antonio et.al. in their study identified general and local factors predisposing to the development of DFUs[24]. The general factors include duration and severity of diabetes, and associated comorbidities such as hypertension, dyslipidaemia, chronic renal disease, peripheral vascular disease and age while the local factors included foot deformity, trauma, callus presence, previous amputation, impaired joint mobility and shoe defects[25].

DIAGNOSIS

Individuals who have diabetes are required to have their neurological, vascular, dermatological, and musculoskeletal conditions evaluated on a yearly basis, at the very least. The American Diabetes Association (ADA) developed a comprehensive foot examination and risk assessment tool that is fast and requires very little specialised medical equipment[26]. Patients who come in exhibiting tissue loss are placed in a higher risk category than those who do not. In situations like these, an evaluation of the
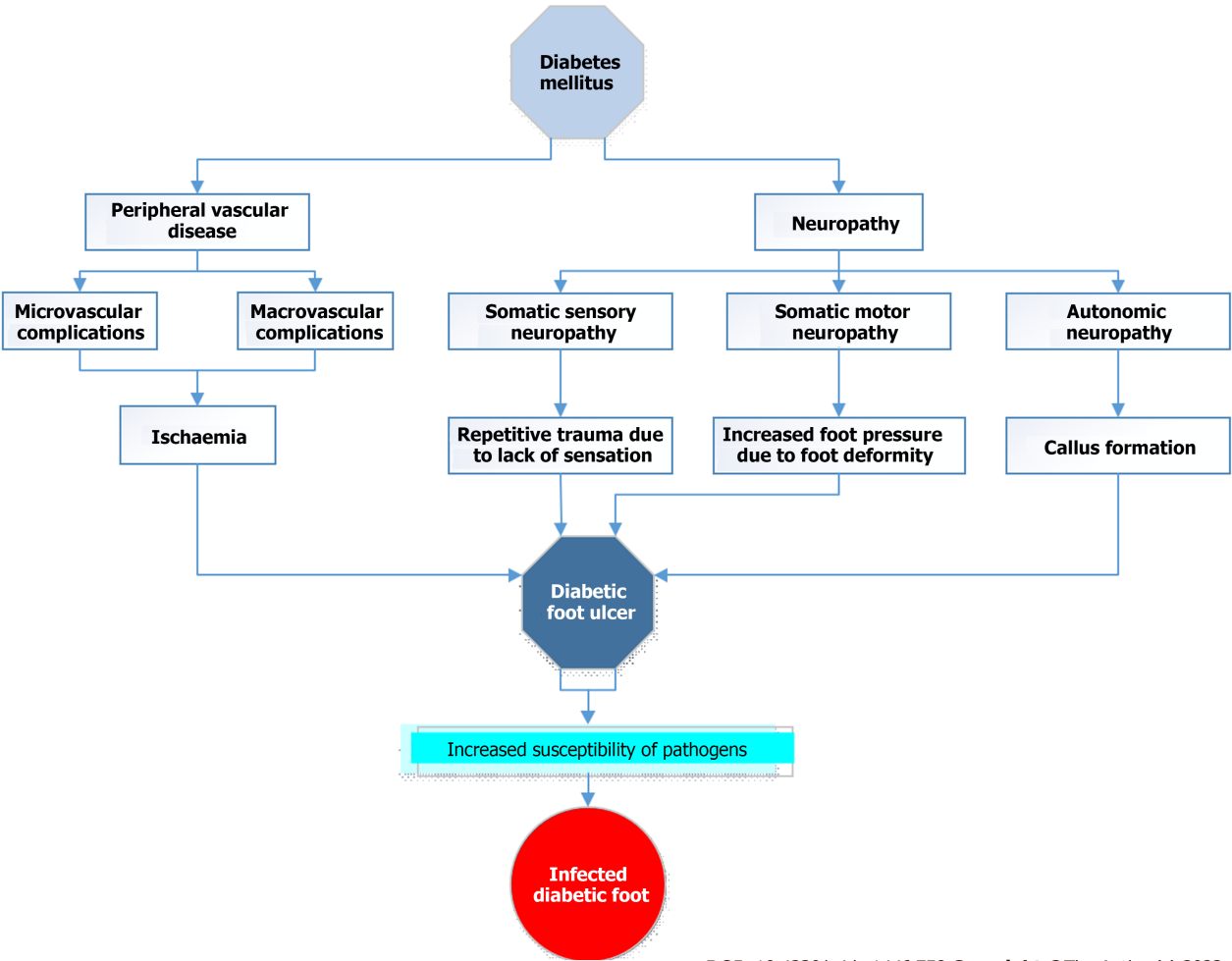
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Figure 1 The common pathways in diabetes mellitus leading to infected foot ulcer.

overall level of limb threat should be performed.

Many measures exist to assess the severity of a diabetic ulcer by analysing the ulcer's features, ischaemia, and infection. Wagner, University of Texas, and PEDIS are the most widely used and globally recognised scales[27,28]. These scales have demonstrated their utility in correlating the degree of severity of the ulcers with the risk of amputation[29]. The wound scales are a valuable tool for classifying the severity of DFUs, but they should not be used to determine the need for amputation. The microbiology of wounds should be examined in each region to further determine the appropriate empiric therapy in the management of DFU.

COMPLICATIONS

DFUs are the major cause of hospitalisation and amputation in diabetes patients[5,25]. Foot ulcer complications include excruciating pain, infection, gangrene, osteomyelitis, amputation, and death[30]. Coexisting diabetes-related problems, such as diminished peripheral sensations and absence of pain along with this sustained ambulation further incite additional damage[31].

Studies demonstrated a higher death rate in diabetic patients with DFUs, with a death rate almost double that of diabetic patients without foot ulcers[22,32]. DFUs have been also reported to be associated with a greater frequency of major cardiovascular risk factors, subclinical signs of past and new-onset cardiovascular and cerebrovascular events [33].

TREATMENT

Current treatment emphasises patient education, regular foot self-examinations, and annual diabetic foot evaluations. These annual examinations comprise patient history, peripheral vascular exam, and sensory nerve function evaluation to detect DPN early. Pressure analysis studies on lowering foot

pressure or changing gait offer promising technology for the early detection and prevention of DFU[34, 35]. Depending on DFU categorization, DFU patients need unloading, infection or ischaemia treatment, wound debridement, and wound dressings[36]. Tissue volume and type are often used to classify DFUs [37]. Granulation tissue is red/pink and symbolises healing tissue, whereas slough tissue is more yellow and represents infected tissue and necrotic tissue is dark/black and shows tissue death. Many studies show that DFU diagnosis and treatment can greatly reduce or prevent serious consequences[37,38]. Despite national and international guidelines, DFU administration varies. Under this ambit, patients suffering from DFUs need reliable and quick therapy, which can only be facilitated with deeper understanding of the metabolic marker of DFU such as advanced glycated end-products (AGE's), inflammatory markers, lipid profile, while newer markers such as adiponectin as a prospective diagnostic tool needs to be further explored. Emerging technologies such as bioprinting and electro-spinning[39], stem and somatic cell monotherapy[40] and grafting techniques[41] offer promising alternatives by overcoming the limitation in conventional approaches.

ADIPONECTIN

Adipose tissue produces adipokines, which are peptides that communicate with other tissues such as the brain, liver, pancreas, immune system, vasculature, and muscle about their functional state. Thus, adipose tissue dysfunction is often related with alterations in the secretion of adipokines such as leptin, adiponectin, fibroblast growth factor 21 (FGF21), retinol-binding protein 4, dipeptidyl peptidase 4, bone morphogenetic protein (BMP)-4, BMP-7, vaspin, apelin, and progranulin. Although the complete repertoire of human adipokines has not yet been described, it has been established that adipose tissue is a reservoir for more than 600 secretory proteins[42].

Adipokines control many physiological processes, including appetite and fullness, fat distribution, insulin secretion and sensitivity, energy expenditure, endothelial function, inflammation, blood pressure, and blood clotting[43,44]. As the mRNA transcript for adipokines was most robustly expressed in adipocytes, adiponectin was first discovered in mice shortly after leptin's discovery in 1995 [45]. Two different adiponectin receptors, ADIPOR1 and ADIPOR2, are responsible for relaying signals from the 30-kilodalton, 244-amino-acid protein, adiponectin, to its target cells[45].

Adiponectin undergoes post-translational modifications that lead to the secretion of oligomers of 90-kDa trimers, which are subsequently detected in the bloodstream as 180-kDa hexamers (low molecular weight)[45,46]. Adiponectin structure consists of trimers, hexamers, and higher order complexes that can be formed in the collagen domain of adiponectin before secretion[47,48].

ADIPONECTIN RECEPTORS

Many different receptors, including adiponectin receptors 1 and 2, play roles in mediating adiponectin's effects[49]. These receptors are functionally dissimilar from G-protein-coupled receptors, primarily due to the fact that their polarity is in the opposite direction. It is projected that they include seven transmembrane sections. large level of functional redundancy appears to exist between the adiponectin receptors, as suggested by both single- and double-knockout mice for the receptors[50]. Although the relative ratios of ADIPOR1 and ADIPOR2 expression in different tissues may differ, in general, both are expressed in a very high proportion of tissues. T-cadherin is the name given to a newly discovered molecule that may be found on the cell surface and possesses a considerable affinity for the protein adiponectin[51]. It is not technically a signalling receptor since it does not have an intracellular signalling domain, even though it is capable of binding adiponectin. T-cadherin is necessary, however, in order for adiponectin to reach its full potential in terms of its cardioprotective effects[52].

SECRETION AND RELEASE

Adiponectin is a secretory protein that is only produced by adipocytes. Constitutively synthesized, it accounts for 0.01%-0.05% of plasma protein, which places it in the range of 2-20 g/mL and makes it a component of plasma that is reasonably abundant. Adiponectin is a protein that is fairly stable in circulation, despite the fact that its plasma half-life is only 45-75 min[53]. Other cell types, such as beta cells in the pancreas and certain cell types in the heart and kidneys, also have a strong affinity for adiponectin and can bind to it. Adiponectin is primarily removed from the bloodstream in the liver, making it an important organ in this process[53]. In spite of the fact that adiponectin is secreted by adipose tissue, circulating levels mysteriously decrease when there is an increase in the amount of central adiposity[54]. Despite this, greater degrees of adiposity in the lower extremities and the truncal region are associated with greater concentrations of adiponectin.

Adiponectin's insulin-sensitizing, anti-inflammatory, and antiapoptotic effects have generated considerable interest[45,55]. In addition, numerous cohort studies in various groups have shown that adiponectin levels are inversely related to either the presence of glucose intolerance or the risk of developing T2DM[56].

EFFECTS OF ADIPONECTIN

On Beta-cell function
The beta cells of pancreatic islets express both ADIPOR1 and ADIPOR2, the two receptors for adiponectin[57,58]. Recombinant adiponectin given to adiponectin-deficient mice shows that it targets beta cells. Adiponectin may enhance glucose-mediated insulin production and promote insulin and related gene transcription[59], however, the effect of adiponectin on insulin release in individuals with normal insulin sensitivity is not well-established[57,60].

On cardiac and renal function
The strong and long-standing correlation between adiponectin levels and the development of cardiovascular disease has been well-documented. Pischon *et al*[61] found in a large cohort that men with high plasma adiponectin levels had a lower risk of myocardial infarction. In preclinical ischaemia/reperfusion trials, the Walsh group showed that recombinant adiponectin strongly improves cardiomyocyte survival[62]. However, why end-stage cardiovascular disease has a significant positive correlation between mortality and high adiponectin levels, unlike early stages, is unknown[63].

A similar scenario exists in the kidney, where low adiponectin levels correlate with albuminuria in both animals and humans[64]. In animal models with adiponectin gene knockout, the lack of adiponectin has been linked to increased podocyte damage and albuminuria, and adiponectin therapy has demonstrated the ability to reverse certain renal dysfunction[65]. In patients with chronic kidney disease, adiponectin levels are positively correlated with proteinuria[31]. This upregulation is similar to that seen in cardiovascular disease, particularly end-stage cardiovascular disease. The mechanisms are unknown. This is especially challenging given that adiponectin is not cleared through the kidney except in cases of severe proteinuria. This is especially challenging given that adiponectin is not cleared through the kidney except in cases of severe proteinuria[66], making it difficult to determine which mechanisms are responsible.

On insulin sensitivity
Skeletal muscle is an important factor in insulin sensitivity because it is the primary source of glucose for the body as a whole. It should not come as a surprise, consequently, that a substantial amount of attention has been paid to the potential metabolic effects that adiponectin has on this tissue. High-molecular-weight adiponectin correlated better with systemic insulin sensitivity than low-molecular weight in rodents and humans[45,46]. Skeletal muscle has a high concentration of ADIPOR1, through which adiponectin regulates energy metabolism[67]. Most investigations into the effects of adiponectin have focused on its binding to globular adiponectin, which exhibits greater binding strength and biological activity in skeletal muscle compared to most other tissues[68,69]. Adiponectin binding leads to increased glucose uptake and nonoxidative glycolysis, while simultaneously reducing intramyo-cellular triacylglycerol content and enhancing fatty acid oxidation[68,69]. Additionally, adiponectin influences the number of mitochondria and the types of oxidative fibers present in skeletal muscle[70]. However, in diseased states, the effects of adiponectin on skeletal muscle are attenuated.

The liver is affected in a number of different ways by adiponectin. One of the most notable effects is hepatic glucose production inhibition, which lowers body glucose levels. Hepatocytes are insulin-sensitive at physiological adiponectin levels. As a result, glucose production is significantly inhibited in response to any given dose of insulin[50]. Adiponectin inhibits both the expression[68,71] and activity of important regulators in the process of gluconeogenesis[71,72]. Studies using murine euglycemic clamps have shown that the rates of glucose disposal, glycolysis, and glycogen synthesis are not affected by the presence of intravenous adiponectin infusion[72]. This suggests that the primary mechanism by which adiponectin lowers blood sugar levels is through the suppression of hepatic glucose output, rather than through enhancing glucose disposal.

On adipose tissue
The adiponectin receptors (ADIPOR1 > R2) are also reported to be expressed by adipocytes. This data further implies that adiponectin may alter adipose tissue function locally, either with modifying autocrine or paracrine function.

As anti-inflammatory effector
Adiponectin's impact on inflammation is not limited to adipose tissue, and its anti-inflammatory effects have been observed in other contexts. This is significant because systemic inflammation is thought to

play a role in the development of insulin resistance[73]. These researchers have shown that adiponectin can inhibit the development and proliferation of bone marrow-derived granulocyte and macrophage progenitors, but it does not have this effect on other haematopoietic cell lines. In addition, it is also reported that inflammatory processes in macrophages can be disrupted, by suppressing the phagocytic activity in human macrophages that have been treated with adiponectin [73], as is the production of pro-inflammatory cytokines[73]. In the setting of the development of atherosclerosis, adiponectin is shown to limit the transition of macrophages into lipid-laden foam cells[74].

On other tissues

Adiponectin works in the brain to increase the amount of energy that is expended, which might lead to weight reduction[46]. In clinical research, circulating adiponectin has been shown to have an independent and unfavourable relationship with components of metabolic syndrome. These components include insulin resistance, body weight, blood pressure, and serum lipids[43,55].

Adiponectin's molecular functions imply that the molecule or agonists of its receptors might cure obesity and related comorbidities[45]. Studies showed that adiponectin improved insulin sensitivity, glucose metabolism, insulin secretion, and body weight in rodent models[75]. Recently, it was shown that a synthetic small molecule adiponectin receptor agonist, known as "AdipoRon", greatly increased insulin sensitivity and decreased glucose intolerance in rats[76]. AdipoRon treatment prolonged the lives of high-fat-fed db/db mice, adding support to the idea that higher blood adiponectin levels are associated with a later average age of mortality in obese people[76]. Levels of adiponectin have been shown to have a negative correlation with obesity, visceral fat, T2DM, and other complications that are associated with obesity[45,55]. Not only adiponectin has a promising and readily detectable stable marker for a variety of illnesses, but it also has the potential to play a big role in the future of clinical importance as a therapeutic agent. This is because adiponectin has the ability to regulate fat storage[44].

The potential therapeutic role of adiponectin in DFUs

Many studies have shown that diabetic patients have lower adiponectin levels than healthy controls[77-79]. Adiponectin deficiency has been correlated with increased susceptibility to diabetes and its associated complications, such as DFUs[80,81]. Some investigations have also indicated that low adiponectin levels may be a potential biomarker of poor wound healing and increased amputation risk in diabetic foot ulcer patients[78,81,82]. Figure 2 depicts the probable mechanisms *via* which reduced adiponectin levels may contribute to the development of DFUs. Nonetheless, more research is needed to completely understand the role of adiponectin in the pathophysiology and therapy of DFUs.

ADIPONECTIN AND KERATINOCYTES

Diabetic patients often experience impaired wound healing due to continuous hyperglycaemia, leading to alterations in various stages of the healing process such as haemostasis, inflammation, proliferation, and remodelling. Factors that contribute to this include hypercoagulability, impairment of skin function [83], imbalanced inflammatory and growth factors[84], reduced neutrophil function[85], and insufficient wound re-epithelialization[86]. Adipose tissue has recently been recognized as a key endocrine organ with a role in wound healing. This role is due to the secretion of bioactive substances known as adipokines, which regulate paracrine signaling[87]. There is evidence from numerous research that adipose tissue contributes to the healing of wounds[88-90]. Despite the current understanding, the exact role of adipocytes in the wound healing process remains unknown. However, it has been demonstrated that applying adipose tissue extracts over skin wounds can improve wound repair[91]. The healing process is believed to take place *via* paracrine signaling, highlighting the significance of the adipokines released by adipose tissue in wound healing[92].

Adiponectin, that is secreted from adipocytes has been found to aid in wound healing through its effects on keratinocytes, the most abundant cellular component of the epidermis[93]. Adiponectin promotes keratinocyte proliferation and migration, which is crucial for proper re-epithelialization and wound closure. This is mediated *via* the AdipR1/AdipR2 and ERK signaling pathways[94]. Adiponectin also elevates the intracellular and reconstructed epidermal lipid content of keratinocytes, and regulates the expression of lipid biosynthesis enzymes and nuclear hormone receptors, which helps maintain skin barrier integrity, an action that is mediated through SIRT1 signaling molecule (SIRT1)[95].

Furthermore, adiponectin possesses reactive oxygen species (ROS)-scavenging abilities and can mitigate oxidative stress-induced DNA damage while regulating antimicrobial peptide production in senescent keratinocytes[96-98]. Studies have shown that adiponectin can reverse premature cellular aging in keratinocytes and restore normal antimicrobial peptide levels by activating AMP-activated protein kinase (AMPK), increasing SIRT1 deacetylation, recovering FoxO1 and FoxO3 transcription activity, and suppressing NF-κB p65, thereby preventing abnormal expression of human β-defensin 2 induced by hydrogen peroxide[99]. Additionally, it restores filaggrin expression and normalizes keratinocyte activity, which is crucial for maintaining skin integrity as an immune barrier[100,101]. Therefore, one way in which adiponectin may promote DFU healing is through its impact on skin
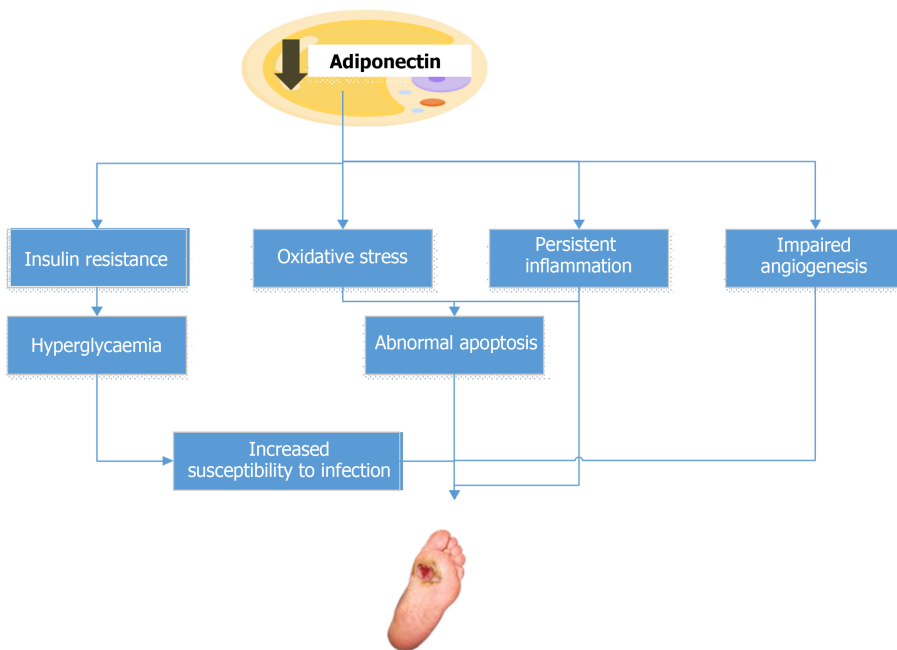
Figure 2 The role of low adiponectin in contributing to delayed wound healing.

integrity, keratinocyte proliferation, and migration. However, further research is necessary to fully understand the potential mechanisms of adiponectin in DFU healing.

ADIPONECTIN AND EXTRACELLULAR MATRIX REMODELLING

The extracellular matrix (ECM) that is produced, assembled, and remodeled by fibroblasts is crucial for maintaining skin integrity, but when it is damaged, as in skin ulcers, it undergoes repair and remodeling. Matrix metalloproteinases (MMPs), a family of proteins that includes collagenases and gelatinases, are ECM enzymes that break down damaged fibrils during ECM remodeling[102]. Normal ECM remodeling includes a delicate balance of ECM breakdown, generation, and maturation. In poor wound healing, such as DFU, the process of ECM remodeling tends to yield more degraded, non-soluble fibrils, resulting in a disorderly ECM network and callus formation[103,104].

Abnormal expression of MMPs and differential expression of ECM contribute to poor healing in DFUs[105-107]. Elevated MMP activity and imbalanced tissue inhibitors of metalloproteinases (TIMPs) have been reported in the skin of diabetic ulcer patients. A study reported that enhanced expression of MMP-1 is necessary for wound healing in DFU, while enhanced MMP-8 and MMP-9 contribute to delayed wound healing. Furthermore, a higher MMP-1/TIMP-1 ratio may indicate a proteolytic environment in the wound[106,107]. Adiponectin has been found to suppress fibroblast proliferation, migration, and ECM formation[108], as well as increase the expression of fibroblasts and type 1 collagen components of the ECM[109,110]. The endogenous expression of adiponectin and its malfunctioning may play a fundamental role in skin fibrosis and exert a substantial negative regulatory impact on fibrosis[111].

In summary, adiponectin has been shown to influence ECM composition by regulating the activity of ECM-associated molecules, such as collagen, elastin, and glycosaminoglycans, implicating that as a potential mechanism through which adiponectin may help promote DFU healing.

ADIPONECTIN'S ANTI-INFLAMMATORY PROPERTIES AND WOUND HEALING

Another contributing factor to poor wound healing in diabetic patients is the presence of excessive inflammatory reaction[112,113]. DFUs which are characterized by chronic inflammation and infection can lead to tissue necrosis and amputation[114-116]. In individuals with diabetes, the wound healing process is often hindered as the wounded tissues remain in the late inflammatory phase. In such cases, macrophages are unable to transition into the repair phenotype and release the necessary factors that promote tissue repair. As a result, the wound fails to progress from the inflammatory to the proliferative phase of healing, leading to persistent inflammation[117]. The persistent inflammation in DFUs is

contributed by the activation of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, as well as the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway[112,113,118]. In addition, the downregulation of neuropeptides that are essential for wound healing and biofilm development also contributes to the persistent inflammatory state in DFUs. Biofilms, which disrupt wound healing by creating a prolonged inflammatory response, limit macrophage phagocytosis and keratinocyte growth migration, and transmit antimicrobial resistance genes[119-121].

Adiponectin has an anti-inflammatory effect and is a potential therapeutic option for preventing and treating DFUs. It has been demonstrated that adiponectin reduces the expression of pro-inflammatory markers and inhibits the activation of the NF-κB signaling pathway in human aortic endothelial cells and monocytes[122-124]. Activation of AdipoR1 and AdipoR2 receptors increases the activity of AMPK and inhibit the NF-κB signaling in various cells including macrophages, liver, and skeletal muscle. Both contribute to adiponectin's anti-inflammatory properties[50,125]. The crucial role of AMPK signaling activity in wound healing is highlighted by the successful improvement of DFU healing achieved through the reactivation of AMPK signaling[126]. Adiponectin may also inhibit the activation of the inflammasome, a complex of proteins which plays a key role in the inflammatory response[127,128].

Adiponectin stimulates the nuclear receptor peroxisome proliferator-activated receptor-gamma (PPAR-γ), which affects glucose and lipid metabolism[129,130]. When PPAR- is activated, pro-inflammatory cytokines are suppressed, and anti-inflammatory genes are activated. Adiponectin also suppresses the creation of reactive oxygen species and the activation of NADPH oxidase[131], which contribute to inflammation and oxidative stress. Adiponectin may also limit the migration and proliferation of vascular smooth muscle cells, as well as the development of new blood vessels[132-134], while promoting the regression of existing blood vessels, which can also contribute to its anti-inflammatory effects[135-137]. Hence, adiponectin's anti-inflammatory properties may aid wound healing by minimising prolonged inflammation and accelerating the wound's transition into the proliferative phase of recovery.

ADIPONECTIN AS AN ANTIBACTERIAL IN DFUS

A meta-analysis by Macdonald *et al*[138] found that DFUs are caused by several genera of bacteria, mainly gram-positive. Another study by Smith *et al*[139] revealed populations of gram-positive bacteria and both aerobes and anaerobes. These bacteria can form biofilms, making it more difficult for antimicrobials to access and thus slowing down the healing process[140].

Diabetics are also susceptible to periodontitis, which is associated with dysbiotic plaque biofilms and eventually leads to the destruction of the tooth-supporting structures. DFUs are similar in that they comprise bacteria that form biofilms and eventually lead to destruction of the underlying bone structures. A study by Wang *et al*[140] suggested that the level of adiponectin has an inverse association with periodontitis. Treatment with adiponectin in animal experiments better improved tissue destruction and suppressed inflammation, which improved bone regeneration[141]. There is little literature on the use of adiponectin as an antibacterial for DFUs. However, a study by Wang *et al*[140] suggests that adiponectin may inhibit inflammation stimulated by obesity or by periodontal pathogens and somehow influence antibacterial outcomes.

Given these findings, further research is needed to explore the antibacterial effects of adiponectin in DFUs and its use as a candidate for the treatment of this chronic condition.

ADIPONECTIN AND IMMUNE RESPONSE

One contributing factor to the delayed healing and susceptibility to bacterial infection in DFUs is the low immune response. Research has shown that adiponectin has the ability to modulate immune cell activity by inhibiting the activation and differentiation of T-helper 1 (Th1) cells, which leads to the emergence of inflammatory and autoimmune diseases, while promoting the activation and differentiation of Th2 cells, which regulate immune responses[142]. Adiponectin achieves its anti-inflammatory effects by regulating multiple signaling pathways and modulating cellular processes involved in inflammation, making it a promising therapeutic target for various inflammatory and metabolic disorders. Additionally, studies suggest that adiponectin can modulate bacterial infection by regulating the activity of molecules responsible for bacterial uptake and killing[143,144].

ADIPONECTIN AND FIBROBLAST GROWTH FACTORS IN DFU HEALING

Fibroblast growth factors (FGFs) are proteins that are expressed in various tissues and play a crucial role in wound repair[145]. There are two types of FGFs: Paracrine and endocrine. Endocrine FGF regulates

various metabolic processes and cell survival, while paracrine FGFs regulate neural development, angiogenesis, and wound healing. Studies have shown that specific types of FGFs for instance aFGF, bFGF, and the FGF 15/19 subfamily may have a positive effect on diabetic wound healing. aFGF aids in diabetic ulcer healing by stimulating capillaries, fibroblasts, and proliferative proteins in ulcer tissue [146]. Regulating the release of bFGF has also been shown to enhance skin wound healing and epithelium development in diabetic mice, while also minimizing scar formation by promoting fibroblast and myofibroblast apoptosis[147]. Additionally, FGF-19 and FGF-21 have been found to be excessively expressed in the serum of diabetes patients[148,149]. FGFs are more potent angiogenesis factors than platelet-derived growth factor and vascular endothelial growth factor (VEGF). FGFs enhance the development of granulation tissue by increasing fibroblast proliferation and angiogenesis[150].

ADIPONECTIN AND ANGIOGENESIS

Studies have shown that impaired angiogenesis contributes to the poor healing of DFUs[151,152]. This is due to various factors such as the failure of macrophages to change into a repair phenotype[151], elevated levels of plasma pigment epithelium-derived factor (PEDF), and dysregulation of angiopoietin-1 (Ang 1) and Ang 2. Macrophages are a key source of VEGF and other pro-angiogenic substances in wounds. On the other hand, PEDF has been found to delay wound healing and decrease angiogenesis in diabetic wounds[152].

Adiponectin has both pro-angiogenic and anti-angiogenic effects, depending on the signaling pathways involved. Adiponectin can promote the formation of new blood vessels through various mechanisms. For example, it increases the production of pro-angiogenic factors like VEGF and FGF-2, and stimulates the migration, proliferation, and differentiation of endothelial cells. This is thought to happen because adiponectin activates signaling pathways like Akt and AMPK[153,154]. However, adiponectin can also inhibit angiogenesis in some contexts. It decreases the production of pro-angiogenic factors and inhibits the expression of angiogenic factors like FGF-2. Additionally, it can inhibit the migration and invasion of certain cancer cells through the modulation of signaling pathways like Akt and AMPK[155].

Hence, the effects of adiponectin on angiogenesis could help promote wound healing. However, those effects are context-dependent and complex. Further research is needed to understand the molecular mechanisms behind these effects and to determine its potential therapeutic applications in different contexts.

ADIPONECTIN AND APOPTOSIS

Impaired apoptosis is another factor that contributes to the poor healing of DFUs[156]. During the wound healing process, different cell groups go through various stages of clearance, culminating in apoptosis. DFU trauma causes mitochondrial damage, which increases the expression of pro-apoptotic proteins while decreasing the expression of anti-apoptotic proteins such as B-cell lymphoma-2 (Bcl-2). This results in apoptosis in cells such as fibroblasts and vascular smooth muscle cells. Low expression of FGF-2, a factor related to fibroblast mitosis and cell survival, has been observed in diabetic wound cells. Reduced expression of other factors related to fibroblast regeneration, such as adiponectin, also contributes to this process[157].

Furthermore, delayed apoptosis has been reported during the inflammatory phase of wound healing in diabetic mice, which may contribute to the persistent inflammatory state in DFUs[158]. Excessive cell death due to hyperglycaemia can lead to poor structural recombination and difficulty in generating granulation tissue, making the wound more susceptible to infection[156]. In addition, chronic hyperglycaemia associated with altered lipid and glucose metabolism promotes a condition of oxidative stress, which results in long-term chronic inflammation of wounds across all stages of wound healing.

Adiponectin has been shown to have anti-apoptotic effects, which may prevent cell death in the wound area and promote wound healing. Studies have shown that adiponectin can inhibit the activation of the intrinsic apoptotic pathway, leading to the prevention of cell death and promotion of wound healing. For example, a study showed that treatment with recombinant human adiponectin promoted wound healing in diabetic mice by inhibiting the activation of the intrinsic apoptotic pathway. However, further research is needed to understand the molecular mechanisms behind adiponectin's effects on apoptosis and its potential therapeutic applications in different contexts[159].

ADIPONECTIN AND WOUND CONTRACTION

Adiponectin has been identified as a mediator of wound contraction, a process that involves the reduction of wound size through the convergence of wound edges. This action is considered to occur *via*

a variety of molecular mechanisms, including collagen synthesis stimulation, MMP inhibition, and myofibroblast migration and proliferation boosting.

Adiponectin has been found to increase the production of collagen; a critical component of the ECM necessary for wound healing. This is achieved through the activation of the Transforming Growth Factor-β (TGF-β) signalling pathway, which stimulates collagen synthesis *via* the phosphorylation of Smad2 and Smad3. Adiponectin also enhances the activity of procollagen type I and III mRNA, which are necessary for collagen synthesis[160].

Additionally, adiponectin suppresses MMPs, enzymes that degrade the ECM and hinder wound healing. By decreasing the expression of MMP-2 and MMP-9 and increasing the expression of TIMP-1, an inhibitor of MMPs, adiponectin promotes the maintenance of the ECM[161,162].

Furthermore, myofibroblasts play a crucial role in wound healing and contraction. However, excessive myofibroblast proliferation during the late stage of wound healing can lead to the formation of pathological scars that greatly reduce the quality of wound healing[163]. Studies have shown that adiponectin may prevent the formation of pathological scars by inhibiting myofibroblast synthesis, proliferation, and migration[164,165].

Therefore, adiponectin may increase wound contraction by increasing collagen synthesis, inhibiting MMPs, and modulating myofibroblast migration as well as proliferation. More research is needed to understand the molecular mechanisms of these effects and to assess the therapeutic potential of adiponectin in wound healing.

ADIPONECTIN AND OXIDATIVE STRESS

Excessive oxidative stress is a hallmark of diabetic wounds, where high levels of ROS are present. The balance between ROS creation and elimination is crucial for proper wound healing. In diabetes, high glucose levels lead to an increase in energy metabolism substrates, which, in turn, result in elevated levels of superoxide and oxidative stress. This increased oxidative stress enhances the production of advanced glycation end products (AGEs)[166,167]. Moreover, nitric oxide synthase decoupling in diabetes leads to decreased nitric oxide production[168], further complicating the healing process. These findings highlight the crucial role that oxidative stress plays in diabetic wound healing and the need to address this issue to improve therapeutic outcomes.

Adiponectin has demonstrated wound healing benefits through its antioxidant properties. Specifically, adiponectin has been shown to increase insulin release[75], enhance insulin sensitivity [169], promote glucose uptake[68,170], and scavenge ROS[171]. These antioxidant properties of adiponectin provide new avenues for the development of effective therapeutic strategies for diabetic wound healing.

According to a review conducted by Woodward *et al*[172], adiponectin has additional anti-inflammatory, anti-apoptotic, and antioxidative effects that can reduce cardiovascular oxidative stress. Matsuda and Shimomura[173] also suggested that adiponectin may protect against oxidative-stress-induced damage in the cardiovascular system, and that circulating adiponectin levels and increased oxidative stress may contribute to the pathogenesis of obesity-associated metabolic diseases. Nguyen [174] proposed that adiponectin could be explored as a focus of new treatment strategies in various metabolic diseases due to its antioxidative, anti-inflammatory, and anti-fibrotic effects, which help regulate glucose levels, lipid metabolism, and insulin sensitivity. However, further research is needed to investigate the antibacterial effects of adiponectin in DFUs and its potential use as a treatment strategy.

ADIPONECTIN AND NERVE FUNCTION

The development and poor healing of DFUs are influenced by peripheral neuropathy, a complex and multi-factorial condition. Among the identified contributors to DPN are oxidative stress, hypoxia, AGEs, activation of T lymphocytes, and insufficiency of nerve growth factors. Reduced expression of neuropeptides is a hallmark of neuropathy in both autonomic and sensory nerve fibers that arise from diabetes mellitus. These neuropeptides, which act as neuromodulators, play a crucial part in the process of diabetic wound healing[175]. Adiponectin has been suggested to promote wound healing in diabetics through its neuroprotective role, although further research is required to fully understand the underlying mechanisms involved[176].

ADIPONECTIN AND INSULIN SENSITIVITY

Persistent hyperglycaemia in diabetic patients is a main factor contributing to delayed wound healing and progression of DFUs[177]. Several studies reported the beneficial effect of adiponectin on insulin sensitivity through its metabolic effects on various tissues, including skeletal muscle, liver, and adipose

tissue. Skeletal muscle, which is a key factor in insulin sensitivity and glucose metabolism, has a high concentration of adiponectin receptors and has been shown to have increased glucose uptake and decreased intramyocellular triacylglycerol content in response to adiponectin binding[178,179]. The liver, on the other hand, experiences decreased glucose production and increased insulin sensitivity when physiological levels of adiponectin are present[180]. Adiponectin has also been shown to have anti-inflammatory effects in various tissues, including adipose tissue and liver[181-184]. In addition, adiponectin has been linked to weight reduction and improved insulin sensitivity, glucose metabolism, and insulin secretion in rodents as evidenced by a recent study[185]. In addition, adiponectin improves insulin sensitivity through modulating the gut microbiome[186].

Thus, adiponectin has been proposed to have potential therapeutic and preventive applications in DFUs through various mechanisms, as outlined in Figure 3.

ADIPONECTIN LEVELS IN DFUS: A COMPREHENSIVE REVIEW OF CURRENT EVIDENCE

To review the available evidence on the measurement of adiponectin levels in DFUs in patients with T2DM, a comprehensive search of relevant databases such as PubMed and Google Scholar was conducted to identify relevant studies. The findings of seven selected studies are presented chronologically in Table 1. The results of these studies revealed a consistent pattern, with lower plasma levels of adiponectin found in patients with DFUs compared to those without DFUs. A negative correlation between the duration of diabetes and the development of DFUs was also observed. The findings further indicated a positive association between low plasma levels of adiponectin and DFUs, and that low adiponectin levels could serve as a predictor for DFUs. The results of these investigations imply that reduced levels of adiponectin in the blood of individuals with T2DM and DFUs may play a role in the emergence of foot ulcers by means of microvascular and inflammatory processes.

ROLE OF ADIPONECTIN IN WOUND HEALING AND METABOLIC CONDITIONS: INSIGHTS FROM *IN VITRO* AND *IN VIVO* STUDIES

Adiponectin has been found to play a crucial role in wound healing, both *in vivo* and *in vitro*. Kumada *et al*[187] found that the incubation of human monocyte-derived macrophages with human recombinant adiponectin increased tissue inhibitor of metalloproteinases; TIMP-1 mRNA levels in a dose-dependent manner without affecting MMPs mRNA levels. Adiponectin selectively increased TIMP-1 expression in human monocyte-derived macrophages through the induction of IL-10[187].

Kawai *et al*[188] investigated the effect of human recombinant adiponectin on an immortalized human keratinocyte cell line (HaCaT) and found that adiponectin suppressed the gene expression of involucrin, a marker of keratinocyte differentiation, in a dose-dependent manner. Adiponectin also upregulated the expression of TGFb1 in HaCaT cells and promoted apoptosis in keratinocytes, which could inhibit hyperkeratosis during wound healing in diabetic patients[188,189].

Shibata *et al*[94] found that adiponectin was a powerful mediator in the regulation of cutaneous wound healing. Adiponectin receptors were found in normal human keratinocytes, and adiponectin increased keratinocyte proliferation and migration *via* AdipoR1/AdipoR2 and the ERK signaling pathway. Wound closure was significantly delayed in adiponectin-deficient mice compared to wild-type mice, and both systemic and topical adiponectin treatment improved wound healing in adiponectin-deficient and diabetic mice[94].

In 2013, an orally active adiponectin receptor agonist, AdipoRon, was developed and was found to have similar effects to adiponectin[76,190], improving insulin sensitivity and glucose tolerance, lipid metabolism[190], and vascular dysfunction in type 2 diabetic mice[192].

Salathia *et al*[193] found that the injection of adiponectin into the skin edges of a wound accelerated healing and enhanced epithelialization at the wound margin. Jin *et al*[194] found that adiponectin promoted the growth and migration of preadipocytes in an adipose tissue wound healing study. FGF21 has also been shown to stimulate the production of adiponectin, which could contribute to the expansion of subcutaneous fat and improvement of systemic insulin sensitivity[195].

Kim *et al*[196] conducted a study on the effects of AdipoRon, a synthetic adiponectin receptor agonist, on diabetic nephropathy in T2DM patients. The study found that AdipoRon treatment reversed kidney abnormalities caused by diabetes in mice. The renoprotective benefits of AdipoRon were achieved through the activation of AdipoR1 and AdipoR2 receptors in the kidneys, which improved pathways related to lipid accumulation and endothelial impairment. AdipoRon also increased intracellular Ca^{2+} levels and activated a CaMKK/phosphorylated Ser431LKB1/phosphorylated Thr172AMPK/PPAR pathway, reducing oxidative stress and apoptosis, and improving endothelial dysfunction. The study also found that AdipoRon had cardioprotective benefits through the same mechanism as shown in the kidney[196].

Table 1 Adiponectin levels in patients with diabetic foot ulcers: A summary of published studies

No.	Ref.	Country	Study objective	Study design and sample size	Results	Adiponectin levels (ng/ mL)			*P* value	Conclusion
						Non-Diabetic	Diabetic without FUs	DFU		
1	Tuttolomondo *et al*[203], 2010	Italy	To investigate the plasma levels of adiponectin, resistin and IL-6 in subjects with diabetic foot in comparison with subjects without foot complication	Case-control; sample size: 34 patients with type 2 DM with FU and 37 patients with type 2 DM without FUs	The patients with DFUs exhibited higher CRP, HbA1c, lipid profile, IL-6, resistin and lower levels of adiponectin; DFU patients have lower median; plasma levels of adiponectin; patients with foot ulcers had a longer duration of DM, higher percentage was associated with nephropathy, peripheral artery diseases, ischemic heart diseases, transient ischemic attacks or stroke	NA	8.48×10^3 $(5.15 \times 10^3$ $-12.87 \times 10^3)$[1]	7.145×10^3 $(4.470 \times 10^3$ $-12.170 \times 10^3)$[1]	0.022	Adiponectin levels are negatively correlated with the duration of diabetes and the development of DFUs
2	Zubair *et al* [81], 2012	India	To investigate the association between inflammation and acute foot syndrome	Case-control; sample size: 162 diabetics with FUs & 162 diabetics without FUs	Adiponectin levels were lower in DFU patients than in subjects without DFU; multiple linear regression analysis showed a significant negative correlation between adiponectin levels and DFU (R2 = -0.0189)	NA	13.4 (12.1-14.2)[1]	8.4 (7.1-9.2)[1]	< 0.0001	Diabetic subjects with various grades of diabetic foot ulcer showed a higher IL-6, hsCRP, TNF-α, and lower adiponectin plasma levels in comparison with diabetes without foot ulcer, independent of the concomitant infections
3	Ahmad *et al* [82], 2012	India	To evaluate plasma levels of Cathepsin D, adiponectin, TNF-α, IL-6, and hsCRP in subjects with diabetic foot in comparison with subjects without foot complic-ations	Prospective cohort multicentric hospital-based study; sample size: 211 diabetics with FUs, 208 diabetics without FUs	The median levels of adiponectin were lower in patients with DFUs; adiponectin plasma levels were found to be negatively correlated with various cardiovascular risk factors, including hypertension, dyslipidemia, and microvascular complications such as neuropathy, retinopathy, nephropathy, and PAD; this was found through both multiple linear regression analysis and forward stepwise regression analysis	NA	13.3 (12.1-14.2)[1]	8.5 (7.1-9.5)[1]	< 0.0001	Low plasma adiponectin is a predictor for DFUs; the study suggests that low levels of adiponectin in diabetic patients with foot ulcers could be linked to the development of foot ulcers through microvascular and inflammatory mechanisms. The findings also indicate that adiponectin may play a role in inhibiting the expression of adhesion molecules on endothelial cells, which are involved in the inflammatory vascular response
4	Dhamodharan *et al*[204], 2015	India	To investigate the genetic association of IL-6, TNF-α, and SDF-1 polymorphisms with serum cytokine, adiponectin, leptin and hsCRP levels in diabetic foot ulcers	Case-control; sample size: A total of 515 subjects were divided into four study groups: Group-I (NGT)/control; *n* = 106), group-II known T2DM without DFU (T2DM; *n* = 139); group-III T2DM with neuropathic DFU (DFU-DN; *n* = 191); group-IV T2DM with	The levels of adiponectin were significantly lower in the diabetic groups (T2DM, DFU-DN, and DFU-PVD) compared to the NGT group	536.0 (0.1-1787.0)[2]	528.6 (6.2-1255.0)[2]	524.0 (63.3-1641.0)[2] in DFU+ DN; 453.5 (164.9-1078.0)[2] in DFU + PVD	< 0.05	Low adiponectin levels can be a biomarker of DFUs; SNPs in cytokine/chemokine genes are useful biomarkers for DFU and can help predict the risk of developing DFU

				PVD (DFU-PVD; n = 79)						
5	Viswanathan et al[205], 2018	India	To examine the involvement of IL-6, TNF-α, and SDF-1) polymorphisms in determining the susceptibility to foot microbial infection, grade of the ulcer) and treatment-outcome; (Debridement vs amputation) in DFU subjects and further, the effect of these SNPs on serum cytokine levels and biomarkers such as leptin, adiponectin, CRP and HOMA-IR	Cross-sectional; sample size: 270 DFU subjects	Data on adiponectin levels are not reported	NA	NA	NA	NA	Screening for SNPs in TNF-α, SDF-1, and IL-6; among DFU subjects would help in identifying high risk individuals and might aid in better patient care
6	Anguiano-Hernandez et al [206], 2019	México	To assess the modification in adiponectin, HIF-1α, NF-κB, IGFBP-3, VEGF and adiponectin in diabetic foot ulcers treated with hyperbaric oxygen	Study design: Not specified; sample size: 17 ambulatory patients and one hospitalized; patient with DFUs; 15 were males & 3 females; 17 T2DM and 1 T1DM; grade 3 and 4 on Wagner scale	Adiponectin levels increase after therapy	NA	NA	-14943 ± 7915[2] (before therapy); -17281 ± 7962[2] (after therapy)	0.035	The study found that while treatment increased adiponectin levels, the increase was not significant; however, all patients showed an increase in angiogenesis and fibrosis and a decrease in ulcer size and infection signs after undergoing HBO$_2$ therapy. The results suggest that HBO$_2$ stimulates the expression of IGFBP-3, NF-κB, and HIF-1α and modulates the inflammatory response related to hypoxia
7	Vangaveti et al [207], 2022	Australia	To determine vildagliptin's effect on inflammatory markers and wound healing in patients with type 2 diabetic foot ulcer	Prospective, randomized, double-blind, placebo controlled, single-centre study; sample size: 50 participants; 25 were assigned to the placebo and 25 to the treatment group	Vildagliptin treatment led to significant improvements in key health markers, including reduced HbA1c, hematocrit, total cholesterol, LDL cholesterol, and total/HDL cholesterol ratio compared to the placebo group. Additionally, vildagliptin demonstrated a protective effect on DFU wound healing	NA	NA	11822 ± 2584.0[3]; Placebo; following; treatment 13138 ± 2671[2]	1.0	The vildagliptin treatment in DFU patients improve wound healing with an associated reduction in some inflammatory biomarkers and a non-significant increase in adiponectin

[1]Data presented as median and interquartile (lower and upper quartile).

[2]Data presented as mean ± SD.

[3]Data presented as mean ± SEM.

NA: Not available; DFU: Diabetic foot ulcer; NGT: Normal glucose tolerance; DN: Diabetic neuropathy; PAD: Peripheral artery disease; PVD: Peripheral vascular disease; CRP: C-Reactive protein; hsCRP: High-sensitivity C-reactive protein; HIF-1α: Hypoxia-inducible factor-1α; NF-κB: Nuclear factor-kappa B; IGFBP-3: Insulin-like growth factor-binding protein-3; VEGF: Vascular endothelial growth factor; HBO$_2$: Hyperbaric oxygen; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; SNPs: Single nucleotide polymorphisms; SDF-1: Stromal cell-derived factor; VEGF: Vascular endothelial growth factor; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

Hong et al[95] conducted a study examining the impact of recombinant human full-length adiponectin on human epidermal keratinocyte cell culture. The results showed that adiponectin improved the differentiation and lipid content of keratinocytes through modulation of the expression of multiple enzymes involved in lipid synthesis and regulation within these cells[95].

Adiponectin replacement therapy has the potential to treat various human diseases, but due to the challenges in using the intact protein, efforts have focused on developing peptide and small molecule agonists of the adiponectin receptor. One such example is ADP355, a peptide that has low nanomolar
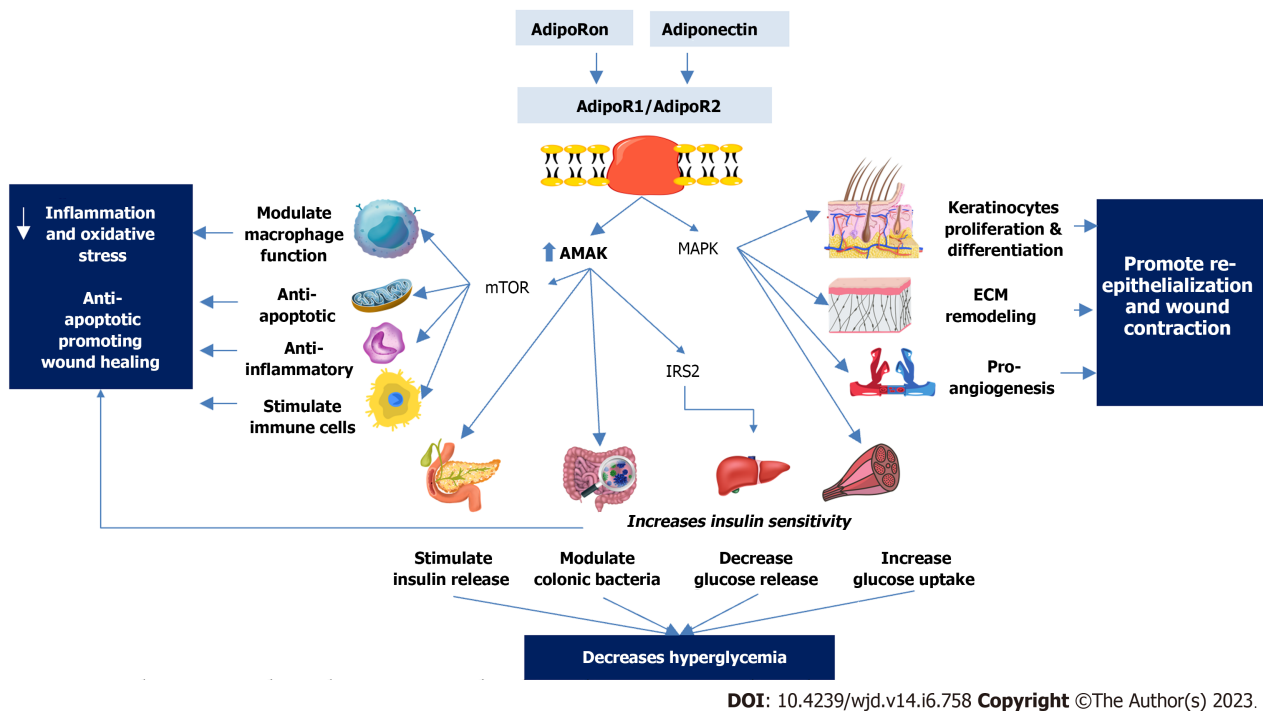
Figure 3 The Potential mechanisms involved in adiponectin-mediated wound healing in diabetic foot ulcers. AdipoRon: Adiponectin receptor agonist; AdipoR1/AdipoR2: adiponectin receptors 1 and 2; MAPK: Mitogen-activated protein kinases; IRS2: Insulin receptor substrate 2; AMAK: Adenosine monophosphate-activated kinase; mTOR: Mammalian target of rapamycin; ECM: Extracellular matrix.

cellular activity and efficacy in treating fibrotic and inflammation-related diseases. On the other hand, small-molecule therapies like AdipoRon can be taken orally and target multiple metabolic conditions. However, the difficulty in comparing the efficacy of different drug classes due to the use of various *in vivo* models and the limitations of *in vitro* measures makes it challenging to determine their effectiveness. Adiponectin receptor antagonists can still be useful in target validation studies, but direct receptor agonists have been shown to be more effective in controlling direct signalling than therapies that aim to increase adiponectin production[197].

Studies have shown the potential benefits of AdipoRon, a small-molecule therapy for multiple metabolic conditions, in improving various aspects of health. A 2020 study by Choi *et al*[192] found that chronic oral intake of AdipoRon improved vascular function in type 2 diabetic mice through an endothelium-independent mechanism. Lindfors *et al*[198] discovered that AdipoRon reduced proinflammatory cytokine expression and improved glomerular inflammation and injury in diet-induced obese mice and cultured podocytes. Sun *et al*[199] showed that AdipoRon reduced inflammation markers and apoptosis, improved mitochondrial function, and accelerated wound healing in aged skin. Zatorski *et al*[200] found that AdipoRon had a gastroprotective effect and reduced inflammation in stomach ulcers. Tarkhnishvili *et al*[201] found that AdipoRon changed myocardial substrate preference towards higher glucose consumption in type 2 diabetic mice, although it was insufficient to enhance cardiac output and efficiency. Li *et al*[202] reported that topical adiponectin was effective in improving clinical signs and reducing inflammation in a mouse model of dry eye or alkali burn, while Baradaran-Rafii *et al*[137] showed that topical adiponectin decreased recent corneal neovascularization in rabbits.

Hence, adiponectin plays a vital role in wound healing, tissue regeneration, and metabolic regulation. It is typically administered through injections or orally *via* an adiponectin receptor agonist, such as AdipoRon. Studies have shown that adiponectin enhances wound healing, keratinocyte differentiation, and improves insulin sensitivity, glucose tolerance, lipid metabolism, and vascular dysfunction in diabetic patients. AdipoRon, a small-molecule therapy, has demonstrated similar effects to adiponectin and can be taken orally, targeting multiple metabolic conditions. Although challenges exist in comparing the effectiveness of various drug classes due to differing *in vivo* models and *in vitro* limitations, direct receptor agonists have shown promise in controlling signaling better than therapies aiming to increase adiponectin production. Overall, adiponectin-based therapies appear to be safe and hold potential for treating a range of human diseases.

AREAS FOR FUTURE RESEARCH

Further research is needed to comprehensively understand the effects of adiponectin on DFUs.

Although the existing literature has shown favourable outcomes, there is a need for a more detailed exploration into the mechanisms underlying adiponectin-mediated promotion of wound healing and tissue regeneration. Research studies should be carried out to establish the safety and efficacy of adiponectin as a therapeutic intervention for DFUs in the clinical setting. Despite positive preclinical outcomes, clinical trials are essential to determine the appropriate dose and treatment schedule, as well as any potential adverse effects. Furthermore, additional research is required to identify subgroups of patients that may benefit the most from adiponectin therapy. This could include examining whether specific genetic or demographic factors influence the effectiveness of adiponectin treatment. Studies should be conducted to identify the optimal delivery method for adiponectin therapy, considering that while the topical application has been successful in some studies, other delivery methods such as injection or implantation may be more efficacious in specific cases. Furthermore, research should be conducted to determine whether adiponectin can be used in combination with other treatments for DFUs, such as antibiotics or growth factors, to enhance wound healing and tissue regeneration. Further investigation is also required into the long-term effects of adiponectin therapy, including its impact on wound recurrence rates and overall wound healing outcomes.

CONCLUSION

In conclusion, the available evidence suggests that adiponectin and its receptors agonist may hold promise as therapeutic targets for the management of DFUs. However, to fully comprehend the role of adiponectin in DFU pathogenesis and treatment, additional research is necessary. Future studies should focus on conducting longitudinal investigations to establish a causal relationship between adiponectin levels and DFU incidence. Moreover, exploring treatment strategies aimed at elevating adiponectin levels in patients with DFUs could provide insights into the potential benefits of adiponectin as a therapeutic target. Investigating the specific cellular and molecular mechanisms underlying the relationship between adiponectin and DFUs is also necessary for a comprehensive understanding of the role of adiponectin in the condition. Additionally, clinical trials that evaluate the efficacy and safety of interventions targeting adiponectin levels in DFU prevention and management are needed. Such research could help to identify novel therapeutic targets for DFUs, ultimately leading to more effective management of the condition.

FOOTNOTES

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Preoperative carbohydrate load to reduce perioperative glycemic variability and improve surgical outcomes: A scoping review

Robert Canelli, Joseph Louca, Ciana Hartman, Federico Bilotta

Robert Canelli, Joseph Louca, Department of Anesthesiology, Boston University School of Medicine, Boston Medical Center, Boston, MA 02118, United States

Ciana Hartman, Department of Anesthesiology, Boston Medical Center, Boston, MA 02118, United States

Federico Bilotta, Department of Anesthesiology, Sapienza University of Rome, Rome 00199, Italy

Corresponding author: Robert Canelli, MD, Associate Professor, Department of Anesthesiology, Boston University School of Medicine, Boston Medical Center, 750 Albany Street Suite 2R, Boston, MA 02118, United States. robert.canelli@bmc.org

Abstract

The detrimental effects of both diabetes mellitus (DM) and hyperglycemia in the perioperative period are well established and have driven extensive efforts to control blood glucose concentration (BGC) in a variety of clinical settings. It is now appreciated that acute BGC spikes, hypoglycemia, and high glycemic variability (GV) lead to more endothelial dysfunction and oxidative stress than uncomplicated, chronically elevated BGC. In the perioperative setting, fasting is the primary approach to reducing the risk for pulmonary aspiration; however, prolonged fasting drives the body into a catabolic state and therefore may increase GV. Elevated GV in the perioperative period is associated with an increased risk for postoperative complications, including morbidity and mortality. These challenges pose a conundrum for the management of patients typically instructed to fast for at least 8 h before surgery. Preliminary evidence suggests that the administration of an oral preoperative carbohydrate load (PCL) to stimulate endogenous insulin production and reduce GV in the perioperative period may attenuate BGC spikes and ultimately decrease postoperative morbidity, without significantly increasing the risk of pulmonary aspiration. The aim of this scoping review is to summarize the available evidence on the impact of PCL on perioperative GV and surgical outcomes, with an emphasis on evidence pertaining to patients with DM. The clinical relevance of GV will be summarized, the relationship between GV and postoperative course will be explored, and the impact of PCL on GV and surgical outcomes will be presented. A total of 13 articles, presented in three sections, were chosen for inclusion. This scoping review concludes that the benefits of a PCL outweigh the risks in most patients, even in those with well controlled type 2 DM. The administration of a PCL might

effectively minimize metabolic derangements such as GV and ultimately result in reduced postoperative morbidity and mortality, but this remains to be proven. Future efforts to standardize the content and timing of a PCL are needed. Ultimately, a rigorous data-driven consensus opinion regarding PCL administration that identifies optimal carbohydrate content, volume, and timing of ingestion should be established.

Key Words: Preoperative carbohydrate load; Glycemic variability; Surgical outcomes; Glucose variability; Blood glucose concentration

Core Tip: Preoperative fasting reduces the risk for aspiration perioperatively; however, it may contribute to intraoperative insulin resistance and glycemic variability (GV). High GV is associated with an increased risk for postoperative complications, including mortality. The administration of a preoperative carbohydrate load (PCL) may reduce perioperative GV and lower the risk for postoperative complications. In this scoping review, we establish the clear negative impact of GV in patients with and without diabetes mellitus in a wide range of clinical settings. However, we are unable to determine from the current body of literature whether a PCL reduces GV intraoperatively and improves surgical outcomes. Future efforts to standardize the content and timing of the carbohydrate load are needed, as well as prospective studies that are designed to evaluate the carbohydrate load effect on GV indices.

INTRODUCTION

The detrimental effects of both diabetes mellitus (DM) and hyperglycemia in the perioperative period are well established and have driven extensive efforts to control blood glucose concentration (BGC) in a variety of clinical settings[1-3].

In critically ill patients, intensive insulin therapy titrated to maintain a BGC of 80-110 mg/dL (4.44-6.11 mmol/L) has been shown to reduce morbidity and mortality[4]. In neurosurgical patients, intensive insulin therapy resulted in reduced postoperative infection rates and shorter intensive care unit (ICU) length of stay[5]. However, efforts to maintain tight glycemic control have often resulted in a significant increase in episodes of hypoglycemia[5,6], a complication that has been associated with an increase in all-cause mortality, cardiovascular death, and death due to infectious disease[7], as well as a prolonged ICU length of stay[8].

It is now appreciated that acute BGC spikes, hypoglycemia, and high glycemic variability (GV) lead to more endothelial dysfunction and oxidative stress than uncomplicated, chronically elevated BGC. This holds true in patients with and without DM[9]. Preoperative fasting is the primary approach to reducing the risk for pulmonary aspiration in the perioperative phase; however, prolonged fasting drives the body into a catabolic state and therefore may increase GV, which can be problematic for patients that have been instructed to fast for at least 8 h before surgery. The stress response to surgery enhances gluconeogenesis and hinders glucose uptakes, further exacerbating GV, *via* the release of stress hormones and immune response suppression[10].

Elevated GV in the perioperative period is associated with an increased risk for postoperative complications, including morbidity and mortality. GV is more pronounced in patients with baseline metabolic disorders such as DM and during certain surgical procedures such as open-heart surgery. Preliminary evidence suggests that the administration of a preoperative carbohydrate load (PCL) to stimulate endogenous insulin production and reduce GV in the perioperative period may attenuate BGC spikes and ultimately decrease postoperative morbidity, however, a data-driven consensus opinion regarding this approach has not been established.

The aim of this scoping review is to summarize the available evidence on the impact of PCL on perioperative GV and surgical outcomes, with an emphasis on evidence pertaining to patients with DM. The clinical relevance of GV will be summarized, the relationship between GV and postoperative course will be explored, and the impact of PCL on GV and surgical outcomes will be presented.

A scoping review was used to map this complex, multidisciplinary topic. It was designed to capture the important facets of emerging evidence pertaining to perioperative GV, PCL, and postoperative

outcomes in patients with and without DM. The methodology of this scoping review was based on the framework of Arksey and O'Malley[11]. A scoping review was chosen to capture a wide range of literature that may have been overlooked or eliminated in a systematic review.

The first step in this scoping review was to establish the clinical implications of high GV and related surgical outcomes by performing a preliminary, non-systematic literature search. The keyword terms searched in MEDLINE/PubMed and Google Scholar search engines for this scoping review included glycemic, glucose, variability, surgery, surgical, outcomes, and postoperative.

After establishing the problem, the research question of this scoping review was developed. The effect of PCL on perioperative GV and postoperative outcomes in patients with and without DM was established as the aim of this study. The keyword search terms used to identify pertinent studies that addressed the topic included PCL, glucose variability, GV, DM, surgery, and surgical outcome.

Articles were screened for relevance based on title and abstract. Relevant articles were read and ranked by all authors individually based on quality of study, pertinence to the aim of the study, impact factor of the journal, and impact index per article score. The impact index per article score was obtained from *Reference Citation Analysis* (https://www.referencecitationanalysis.com/), an artificial intelligence technology-based open multidisciplinary citation analysis database. Authors then conferred to select the final papers to be included in each section of this scoping review. Consideration was given to include articles that were very recently published or felt to be pertinent despite low impact index per article scores.

GLYCEMIC VARIABILITY: CLINICAL RELEVANCE

Hyperglycemia, hypoglycemia and GV are associated with mitochondrial oxidative stress, endothelial cell apoptosis, and inflammatory cytokine release[12]. In this section, the 4 articles listed in Table 1 will identify measurable GV indices and will present the clinical relevance of high GV with respect to morbidity and mortality in patients with and without DM.

A multicenter, retrospective observational study was one of the first to investigate the relationship between GV, rather than hyperglycemia or hypoglycemia, and outcomes and had an impact index per article score of 35. This study analyzed 168837 blood glucose measurements from a cohort of 7049 critically ill patients. Patients were divided into survivors and non-survivors for comparison. Two different indices for GV were measured: The standard deviation (SD) from the mean BGC, and the coefficient of variance (CV) defined as the SD divided by the mean BGC expressed as a percentage. Both SD (1.7 ± 1.3 *vs* 2.3 ± 1.6 mmol/L, $P < 0.001$) and CV (20 ± 12 *vs* 26 ± 13%, $P < 0.001$) were significantly lower for ICU survivors when compared to non-survivors. The two GV indices were independent predictors of ICU and hospital mortality and were stronger predictors of mortality than mean BGC[13].

A single-center, retrospective cohort study of 1246 patients with sepsis aimed to investigate different measures of GV to determine which was the best predictor of in-hospital mortality risk. This article had an impact index per article score of 19.2. Three different indices for GV were measured: Glycemic lability index (GLI), mean amplitude of glycemic excursion (MAGE), and SD from the mean BGC. Although all 3 GV indices were significant predictors of mortality in patients with sepsis, GLI predicted in-hospital mortality [odds ratio (OR) 1.25, 95%CI: 1.20-1.32, $P < 0.001$] better than MAGE (OR 1.12, 95%CI: 1.07-1.18, $P < 0.001$) and SD (OR 1.16, 95%CI: 1.11-1.21, $P < 0.001$). Additionally, with each increasing GLI decile, a higher in-hospital mortality rate was observed. The association of GLI and mortality remained after adjusting for a diagnosis of DM[14].

A retrospective study of 1641 patients with an ICU stay > 2 d aimed to determine the association between GV and outcome measures, including ICU mortality and ICU-acquired infection. GV was assessed using four different indices: SD, CV, GLI, and MAGE. When compared to ICU survivors, ICU non-survivors had higher GV as determined by GLI [75.6 *vs* 50.1 $(mmol/L)^2/h/wk$, $P < 0.001$], CV (23 *vs* 21%, $P < 0.001$), SD (1.7 *vs* 1.4 mmol/L, $P < 0.001$), and MAGE (2.7 *vs* 2.4 mmol/L, $P < 0.001$). Mean BGC was not predictive of ICU mortality (7.0 *vs* 7.0 mmol/L, P value not reported). The predictive ability for mortality was not different between SD, CV, GLI, and MAGE; however, the risk of death increased progressively with each increase in quartile of GLI. When compared to patients without infection, patients with ICU-acquired infection had higher GV as determined by GLI [73.5 *vs* 44.6 $(mmol/L)^2/h/wk$, $P < 0.001$], CV (23 *vs* 20%, $P < 0.001$), SD (1.6 *vs* 1.4 mmol/L, $P < 0.001$), and MAGE (2.7 *vs* 2.3 mmol/L, $P < 0.001$). Mean BGC was not predictive of ICU-acquired infection (7.0 *vs* 7.0 mmol/L, P value not reported). GLI had a better predictive ability for ICU-acquired infections compared to MAGE, CV and SD. In patients without DM, GLI was significantly associated with ICU mortality and ICU-acquired infections, with increasing risk for each quartile increase in GLI. For patients with DM, there was no significant association between GLI and ICU mortality; however, there was an association between GLI and ICU-acquired infection[15].

A prospective observational study of 8894 patients admitted to the surgical ward aimed to investigate the association between GV and clinical outcomes including hospital length of stay, readmission rates, and mortality in patients with and without DM. GV was measured in two ways: SD and CV. Higher SD and CV were both associated with longer hospital length of stay in patients with DM (9 ± 8 *vs* 7 ± 5 d for

Table 1 Glycemic variability: Clinical relevance

Ref.	Patient population	Variability index	Reported results
Egi *et al*[13], 2006	7049 ICU patients, DM included		ICU survivors *vs* ICU non-survivors
		SD	SD: 1.7 *vs* 2.3 mmol/L, *P* < 0.001
		CV	CV: 20 *vs* 26%, *P* < 0.001
Ali *et al*[14], 2008	1246 patients with sepsis, DM included		Mortality crude odds ratio, 95%CI
		GLI	GLI: 1.25, 1.20-1.32, *P* < 0.001
		MAGE	MAGE: 1.12, 1.07-1.18, *P* < 0.001
		SD	SD: 1.16, 1.11-1.21, *P* < 0.001
Donati *et al*[15], 2014	1641 ICU patients, DM included		ICU survivors *vs* ICU non-survivors
		SD	SD: 1.4 *vs* 1.7 mmol/L, *P* < 0.001
		CV	CV: 21 *vs* 23%, *P* < 0.001
		GLI	GLI: 50.1 *vs* 75.6 (mmol/L)2/h/wk, *P* < 0.001
		MAGE	MAGE: 2.4 *vs* 2.7 mmol/L, *P* < 0.001
			No infection *vs* ICU-acquired infection
		SD	SD: 1.4 *vs* 1.6 mmol/L, *P* < 0.001
		CV	CV: 20 *vs* 23%, *P* < 0.001
		GLI	GLI: 44.6 *vs* 73.5 (mmol/L) 2/h/wk, *P* < 0.001
		MAGE	MAGE: 2.3 *vs* 2.7 mmol/L, *P* < 0.001
Akirov *et al*[16], 2019	8894 surgical patients, DM included		Hospital LOS: Low GV *vs* High GV
		SD	DM SD: 7 *vs* 9 d, *P* < 0.001
		SD	No DM SD: 7 *vs* 9 d, *P* < 0.001
		CV	DM CV: 7 *vs* 9 d, *P* < 0.001
		CV	No DM: CV 7 *vs* 9 d, *P* < 0.001
			30 d mortality: Low GV *vs* High GV
		SD	DM SD: 5% *vs* 8%, *P* < 0.05
		SD	No DM SD: 3% *vs* 9%, *P* < 0.05
		CV	DM CV: 5% *vs* 9%, *P* < 0.05
		CV	No DM CV: 3% *vs* 9%, *P* < 0.05

ICU: Intensive care unit; DM: Diabetes mellitus; CV: Coefficient of variance; GLI: Glycemic lability index; MAGE: Mean amplitude of glycemic excursion; LOS: Length of stay; GV: Glycemic variability.

both CV and SD, *P* < 0.001 for both) and without DM (9 ± 8 *vs* 7 ± 6 d for both CV and SD, *P* < 0.001 for both). There was no significant association between GV and readmission rates for both DM and non-DM patients. When compared to the low CV cohort, high CV was associated with increased 30-d mortality in patients with DM (9 *vs* 5%, OR = 1.8, 95%CI: 1.2-2.6) and without DM (9 *vs* 3%, OR = 2.7, 95%CI: 2.1-3.3). Similarly, high SD was associated with increased 30-d mortality when compared to the low SD cohort in patients with DM (8 *vs* 5%, OR = 1.6, 95%CI: 1.1-2.4) and without DM (9 *vs* 3%, OR = 2.7, 95%CI: 2.2-3.4)[16].

In summary, for patients in high acuity settings, elevated GV is associated with worse outcomes including hospital length of stay, readmission rates, and overall morbidity and mortality in patients with and without DM. This holds true for a variety of measured GV indices, including SD, CV, GLI, and MAGE. All GV indices appear to be better predictors of morbidity and mortality than mean BGC.

PERIOPERATIVE GLYCEMIC VARIABILITY AND POSTOPERATIVE COURSE

Due to current preoperative fasting guidelines, stress-induced metabolic changes from surgery, and

coexisting endocrine disorders in a subset of surgical patients, the perioperative period is frequently associated with insulin resistance and high GV[17]. In this section, the 3 articles listed in Table 2 will present the impact of perioperative GV on postoperative morbidity and mortality.

The relationship between GV and surgical outcomes has been studied in cardiac surgery. Abnormal GV may be more pronounced in this surgical population as a result of the elevated stress response associated with cardiopulmonary bypass and increased insulin resistance due to iatrogenic intraoperative hypothermia. A prospective, single center observational study aimed to establish whether GV was associated with major adverse events (MAEs) after cardiac surgery in DM and non-DM patients, and had an impact index per article score of 7.2. A total of 1461 patients undergoing coronary artery bypass grafting with or without valvular surgery were enrolled. All enrolled patients had glycated hemoglobin (HbA1c) measured within 30 d of surgery. Patients were grouped into HbA1c > 6.5% and < 6.5% for comparison, and GV was measured by CV. Major adverse event was a composite primary endpoint that included in-hospital death, myocardial infarction, re-operation, deep sternal wound infection, cardiac tamponade, pneumonia, stroke, or renal failure. Patients that experienced an MAE had higher CV when compared to those that did not have an MAE (24 ± 0.07 *vs* $21 \pm 0.08\%$, $P = 0.001$). Patients with an HbA1c > 6.5% had a higher CV (26 ± 9 *vs* $20 \pm 7\%$, $P < 0.001$) than patients with an HbA1c < 6.5%[18].

A retrospective study of 5058 patients aimed to investigate the relationship between GV and adverse outcomes following total hip and knee arthroplasty and had an impact index per article score of 6. Patients were grouped into tertiles defined by CV for comparison of low variability (first tertile, CV ≤ 11.23%), medium variability (second tertile, CV 11.24%-18.54%), and high variability (third tertile, CV ≥ 18.55%). Adverse outcomes included hospital length of stay (LOS), 90-d mortality, re-operations, periprosthetic joint infections and surgical site infections. Average LOS increased as tertile increased (first 4.6 ± 2.5 d, second 5.6 ± 3.9 d, third 6.5 ± 5.5 d, $P < 0.001$). When compared to patients in the first tertile of CV, patients in the third tertile had an increase in the mortality rate at 90 d (0.4 *vs* 0.1%, OR 3.25, 95%CI: 0.93-11.35, $P = 0.06$), periprosthetic joint infections (0.9 *vs* 0.5%, OR 1.86, 95%CI: 1.10-3.13, $P = 0.02$), surgical site infections (1.4 *vs* 1%, OR 1.49, 95%CI: 1.01-2.21, $P = 0.03$). There was no difference in the re-operation rate between these two groups[19].

A retrospective cohort study of 264 patients investigated the relationship between GV and postoperative outcomes for patients having posterior cervical decompression and fusion. This was a relatively new study in the literature and had a low impact index per article score but was included because of its pertinence to the topic. Patients were grouped into tertiles based on postoperative CV (low < 12.3%, moderate 12.4%-20.7% and high 20.8%-57.9%). Of note, patients with types 1 and 2 DM were included. Measured outcomes included inpatient complications, hospital LOS, 90-d readmission, revision, and surgical site infection rates. There was no significant difference in the overall rate of inpatient complications between the low (12.5%), moderate (17.0%), and high (20.4%) CV tertiles ($P = 0.37$). The average hospital LOS was significantly increased for higher CV tertile (low 3.90 *vs* moderate 5.73 *vs* high 6.06 d, $P = 0.01$). When compared to the low CV tertile, the high CV tertile was associated with significantly increased odds of hospital readmission (OR 4.77, 95%CI: 1.10-6.05, $P = 0.03$) and development of surgical site infection (OR 4.35, 95%CI: 1.09-15.05, $P = 0.04$), but not rates of revision surgery (OR 1.76, 95%CI: 0.70-6.50, $P = 0.19$)[20].

In summary, elevated perioperative GV is associated with increased hospital length of stay and an increased risk for postoperative morbidity and mortality for patients with and without DM. The risk of reoperation does not appear to be associated with elevated GV.

PREOPERATIVE CARBOHYDRATE LOAD: IMPACT ON GLYCEMIC VARIABILITY AND SURGICAL OUTCOMES IN PATIENTS WITH AND WITHOUT DM

Reducing the magnitude of GV has been shown to reduce oxidative stress and systemic inflammatory markers in nonsurgical, diabetic patients[21]. In surgical patients, the administration of a PCL increases endogenous insulin production, reduces the risk of the body entering a catabolic state, and may reduce GV. In this section, the 6 studies listed in Table 3 will present the impact of PCL on GV and surgical outcomes. Notably, early PCL studies, including the first three in Table 3, excluded patients with DM, citing concerns for delayed gastric emptying, increased risk for aspiration, and/or exaggerated BGC response to the PCL. The subsequent three studies were included in this review because they established the safety of PCL administration to patients with type 2 DM.

A single center, randomized controlled trial aimed to determine the effectiveness of a PCL on postoperative nausea and vomiting and postoperative pain in same-day surgery patients. This article had an impact index per article score of 5.0. Patients with DM were excluded. A total of 120 patients scheduled for laparoscopic cholecystectomy were randomized into three groups: 40 patients in the intervention group were instructed to consume one PCL drink [400 mL, 12.5% carbohydrates (CHO), 500 kcal/L] the night before surgery and a half PCL drink (200 mL, 12.5% CHO, 500 kcal/L) 2 h prior to surgery, 40 patients in the placebo group were instructed to drink 400 mL of flavored (0 kcal/L) water before midnight and 200 mL of flavored water 2 h prior to surgery, and 40 patients in the control group

Table 2 Perioperative glycemic variability and postoperative course

Ref.	Patient population	Variability index	Reported results
Subramaniam *et al*[18], 2014	1461 cardiac surgery patients, DM included	CV	No MAE *vs* MAE
			CV: 21% *vs* 24%, *P* = 0.001
			HbA1c < 6.5% *vs* > 6.5%
			CV: 20% *vs* 26%, *P* < 0.001
Shohat *et al*[19], 2018	5058 patients for total joint arthroplasty	CV	1st tertile of CV *vs* 3rd tertile of CV
			Mortality: 0.1% *vs* 0.4%, *P* = 0.06
			PPJI: 0.5% *vs* 0.9%, *P* = 0.02
			SSI: 1% *vs* 1.4%, *P* = 0.03
			Reop: 1.6% *vs* 1.5%, *P* = 0.83
Patel *et al*[20], 2021	264 patients for cervical spine surgery	CV	1st tertile of CV *vs* 3rd tertile of CV
			Complication: 12.5% *vs* 20.4%, *P* = 0.37
			Hospital LOS: 3.9 *vs* 6.06 d, *P* = 0.01
			Readmission: 3.4% *vs* 7.8%, *P* = 0.03
			SSI: 1.1% *vs* 9.5%, *P* = 0.04
			Reop: 0.4% *vs* 3.8%, *P* = 0.19

DM: Diabetes mellitus; CV: Coefficient of variance; MAE: Major adverse event; MI: Myocardial infarction; Reop: Reoperation; DSWI: Deep sternal wound infection; CVA: Cerebrovascular accident; PNA: Pneumonia; PPJI: Periprosthetic joint infection; SSI: Surgical site infection.

adhered to traditional fasting after midnight guidelines. The intervention group reported lower nausea scores 0-4 h postoperatively when compared to the placebo group (0.65 ± 0.70 *vs* 1.30 ± 0.85, *P* < 0.001) and the control group (0.65 ± 0.70 *vs* 1.23 ± 1.10, *P* = 0.009) but no significant difference in nausea between 4-12 h and 12-24 h. The incidence of vomiting at 0-4 h was 17.5% for the intervention group, 42.5% for the placebo group, and 47.5% for the control group which was significantly lower for the intervention group when compared to the placebo group and control group (*P* < 0.001 and *P* = 0.004 respectively). Pain scores were significantly lower in the intervention group when compared to the placebo and control groups at 0-4 h (*P* = 0.001) and 4-12 h (*P* = 0.005)[22].

A large multi-center, randomized, placebo-controlled phase III trial aimed to evaluate the effectiveness of PCL *vs* placebo in preventing postoperative infections after major elective abdominal surgery. This article had an impact index per article score of 13.5. There was no traditional fasting group in this study. Patients with DM and patients with fasting BGC > 125 mg/dL (7 mmol/L) were excluded. A total of 662 patients were enrolled and randomized into two groups: 331 patients in the intervention group were instructed to consume one PCL drink (800 mL, 12.6% CHO, 500 kcal/L) from the night before surgery to 2 h prior to surgery, and 331 patients in the placebo group received 800 mL of water with the same consumption directions. The primary outcome was the occurrence of at least one postoperative infection including superficial or deep wound infection, organ/space infection, urinary tract infection, pneumonia, sepsis, and septic shock. The primary outcome occurred in 16.3% of the intervention group and 16.0% of the placebo group [relative risk (RR) 1.019, 95%CI: 0.720-1.442, *P* = 1.00] which was not significantly different. Secondary outcomes included insulin requirements, antibiotic therapy, total complications, reoperation, ICU LOS, and hospital LOS. BGC was recorded from the first hour after surgery to postoperative day 3 and insulin was administered for BGC > 180 mg/dL (10 mmol/L). Insulin was required in 2.4% of patients in the intervention group and 16.0% of patients in the placebo group (RR 0.15, 95%CI: 0.07-0.31, *P* < 0.001), with a number needed to treat of 7. No other secondary outcomes were significantly different. Notably, no aspiration episodes were observed in either group[23].

A single-center, randomized controlled study aimed to evaluate the effect of PCL *vs* fasting on outcomes in patients undergoing elective craniotomy. This article had an impact index per article score of 3.0. Patients with DM and patients with fasting BGC > 125 mg/dL (7 mmol/L) were excluded. A total of 120 patients were enrolled into two groups: 58 patients in the intervention group were instructed to consume one PCL drink (400 mL, 12.5% CHO, 500 kcal/L) 2 h before surgery and 62 patients in the control group fasted for at least 8 h prior to surgery. The primary outcome was glucose homeostasis defined by BGC measurements from blood samples drawn perioperatively. The BGC was significantly higher in the intervention group upon entering the operating room (6.3 ± 1.6 *vs* 5.6 ± 1.0 mmol/L, *P* =

Table 3 Preoperative carbohydrate load: Impact on glycemic variability and surgical outcomes

Ref.	Patient population	PCL composition and timing	Reported conclusion
Singh *et al*[22], 2015	120 same-day surgery patients, DM excluded	12.5% CHO, 500 kcal/L; 400 mL before MN + 200 mL 2 h before surgery	Intervention *vs* placebo *vs* control
			Nausea score
			0-4 h: 0.65 *vs* 1.30 *vs* 1.23, P = 0.001
			4-12 h: 0.70 *vs* 0.83 *vs* 1.05, P = 0.066
			12-24 h: 0.25 *vs* 0.43 *vs* 0.35, P = 0.257
			Vomit incidence
			0-4 h: 17.5% *vs* 42.5% *vs* 47.5%, P (I-P) ≤ 0.001, P (I-C) = 0.004
			4-12 h: 7.5% *vs* 12.5% *vs* 32.5%, P (I-P) = 0.459, P (I-C) = 0.005
			12-24 h: 0% *vs* 2.5% *vs* 2.5%, P (I-P) = 0.314, P (I-C) = 0.314
			Pain score
			0-4 h: 5.75 *vs* 7.13 *vs* 6.95, P = 0.001
			4-12 h: 3.53 *vs* 4.08 *vs* 4.65, P = 0.005
			12-24 h: 1.95 *vs* 2.08 *vs* 2.25, P = 0.223
Gianotti *et al*[23], 2018	662 patients undergoing elective major abdominal surgery, DM excluded	12.6% CHO, 500 kcal/L; 800 mL between 8 pm and 2 h before surgery	Intervention *vs* placebo
			Composite infection: 16.3% *vs* 16.0%, P = 1.00
			Insulin requirement: 2.4% *vs* 16%, P < 0.001
			Antibiotic therapy: 30.8% *vs* 29.9%, P = 0.87
			Total complications: 28.1% *vs* 28.4%, P = 1.00
			Hospital LOS: 11 *vs* 11 d, P = 0.44
			Aspiration events: 0 *vs* 0, P = 1.00
Liu *et al*[24], 2019	120 patients undergoing elective craniotomy, DM excluded	12.5% CHO, 500 kcal/L; 400 mL 2 h before surgery	Intervention *vs* control
			Preop BGC: 6.3 *vs* 5.6 mmol/L, P = 0.020
			POD3 BGC: 5.6 *vs* 6.3 mmol/L, P = 0.001
			POD3 handgrip: 25.3 *vs* 19.9 kg, P < 0.0001
			POD3 PEFR: 315.8 *vs* 270.0 L/min, P = 0.036
			Postop LOS: 4 *vs* 7 d, P < 0.0001
Talutis *et al*[25], 2020	169 patients with DM2 undergoing elective major abdominal surgery	55 g CHO in 32 oz (946.35 mL), 5.8% CHO; 16 oz (473 mL) before MN + 16 oz 2 h before surgery	Intervention *vs* control
			Preop BGC: 142 *vs* 129.5 mg/dL, P = 0.017
			1st postop BGC: 159 *vs* 173 mg/dL, P = 0.23
			POD1 BGC: 152 *vs* 137.5 mg/dL, P = 0.004
			Intraop insulin: 0-16 *vs* 0-19 units, P =

			0.63
			POD1 insulin: 0-75 *vs* 0-79 units, *P* = 0.09
			Complication rate: 20% *vs* 27%, *P* = 0.65
			Hospital LOS: 2 *vs* 2 d, *P* = 0.38
			Aspiration events: 0 *vs* 0, *P* = 1.00
Suh *et al*[26], 2021	134 patients undergoing bariatric surgery, DM2 included	50 g CHO in 296 mL, 16.9% CHO, 682 kcal/L; 296 mL before MN + 296 mL 3 h before surgery	Intervention *vs* control
			Hospital LOS: 2.0 *vs* 2.1 d, *P* = 0.65
			PONV score: 13.8 *vs* 15.4, *P* = 0.77
			BGC: 140.7 *vs* 135.3 mg/dL, *P* = 0.34
			Antiemetics: 5.3 *vs* 6 doses, *P* = 0.43
			Readmission: 4.7% *vs* 5.7%, *P* = 0.79
			Complication: 3.1% *vs* 4.3%, *P* = 0.72
			Aspiration events: 0 *vs* 0, *P* = 1.00
Lee *et al*[27], 2022	46 patients with DM2 undergoing elective total joint arthroplasty	12.8% CHO, 500 kcal/mL; 400 mL 2-3 h before anesthesia	Intervention *vs* control
			CV: 16.5% *vs* 10.1%, *P* = 0.008
			J index: 25.3 *vs* 18.9, *P* = 0.046
			HOMA-IR: 8.5 *vs* 2.7, *P* < 0.001
			Hospital LOS: 3 *vs* 3 d, *P* = 0.516
			Nausea: 46% *vs* 29%, *P* = 0.402
			Vomiting: 32% *vs* 8%, *P* = 0.066
			Hypotension: 5% *vs* 13%, *P* = 0.609
			Delirium: 18% *vs* 0%, *P* = 0.045
			Wound dehiscence: 9% *vs* 8%, *P* = 0.999
			Pain score at 6 h: 2 *vs* 2, *P* = 0.725

PCL: Preoperative carbohydrate load; DM: Diabetes mellitus; CHO: Carbohydrate; MN: Midnight; LOS: Length of stay; BGC: Blood glucose concentration; Preop: Preoperative; POD: Postoperative day; PEFR: Peak expiratory flow rate; postop: Postoperative; DM2: Type 2 diabetes mellitus; ERAS: Enhanced recovery after surgery; intraop: Intraoperative; PONV: Postoperative nausea and vomiting; CV: Coefficient of variance; HOMA-IR: Homeostasis Model Assessment Insulin Resistance.

0.020); was similar on postoperative days 1 and 2; and was significantly lower on postoperative day 3 in the intervention group (5.6 ± 1.0 *vs* 6.3 ± 1.2 mmol/, *P* = 0.001). Secondary outcomes included handgrip strength, pulmonary function as measured by peak expiratory flow rate, postoperative surgical and nonsurgical complications, and length of stay. Hand grip strength (25.3 ± 7.1 kg *vs* 19.9 ± 7.5 kg, *P* < 0.0001) and peak expiratory flow rate (315.8 ± 91.5 L/min *vs* 270.0 ± 102.7 L/min, *P* = 0.036) were significantly better in the intervention group on postoperative day 3. Postoperative length of stay was significantly reduced in the intervention group (4 *vs* 7 d, *P* < 0.0001)[24].

A retrospective chart review aimed to determine the effects of a PCL as part of an enhanced recovery after surgery (ERAS) pathway on patients with DM. This article had an impact index per article score of 4.0. The intervention group included a total of 80 ERAS patients with DM undergoing bariatric, gastric, pancreatic, and colorectal surgery, and was compared to the control group of 89 non-ERAS patients with DM undergoing similar surgeries from 1 year prior to inception of the ERAS pathway. Patients with a history of type 1 DM were excluded. The patients in the ERAS group were instructed to consume one PCL drink (473 mL, 5.8% CHO) on the night before surgery and another PCL drink on the morning of surgery. The non-ERAS patients adhered to traditional fasting after midnight guidelines. Primary outcomes included perioperative BGC measurements and insulin requirements. Secondary outcomes included development of postoperative complications. The ERAS patients with DM had elevated BGC measurements in the preoperative holding area (142, range 66-392 *vs* 129.5, range 82-316 mg/dL, *P* =

0.017) and on postoperative day 1 (152, range 84-323 *vs* 137.5, range 86-279 mg/dL, *P* = 0.004) when compared to non-ERAS patients with DM. Intraoperative BGC and postoperative BGC on days 2-5 were not different. Intraoperative and postoperative insulin administration did not differ between the two groups. The complication rates and hospital length of stay were not significantly different. None of the patients experienced an aspiration event[25].

A single center, randomized controlled trial aimed to characterize the impact of PCL administration on postoperative outcomes in bariatric surgery. This article had an impact index per article score of 2.0 but was felt to contribute significantly to the body of literature in this scoping review. Patients with DM were included in this study. A total of 134 patients were enrolled and randomized into 2 groups: 64 patients in the intervention group were instructed to consume one PCL drink (296 mL, 16.9% CHO, 682 kcal/L) on the night before surgery and another PCL drink 3 h before surgery and 70 patients in the control group adhered to traditional "nothing by mouth" after midnight prior to surgery fasting guidelines. The primary outcome was a clinically significant reduction in hospital length of stay. Secondary outcomes included postoperative nausea and vomiting (PONV), postoperative BGC, antiemetics received, hospital readmission rates, and overall complications amongst other outcomes. There was no significant difference noted in hospital length of stay between the intervention and control groups (2.0 ± 1.2 *vs* 2.1 ± 0.9 d, *P* = 0.65). Additionally, there was no significant difference between the two groups with regards to PONV scores, postoperative BGC measurements, antiemetics received, hospital readmission rates, or postoperative complication rates. Notably, none of the patients experienced aspiration during induction of anesthesia[26].

A single center, randomized control trial investigated the effects of PCL on perioperative GV, gastric volume, and postoperative outcomes in patients with DM undergoing elective total knee and hip arthroplasty. This article was recently published and so has not had a significant amount of time to be included as a citation in other works. A total of 46 patients were included in the final cohort of this study. Patients were randomized into 2 groups: 22 patients in the intervention group were instructed to consume one PCL drink (400 mL, 12.8% CHO, 500 kcal/L) 2-3 h before anesthesia and 24 patients in the control group adhered to traditional fasting after midnight guidelines. The primary outcome was GV measured by CV and J index (0.001 × [mean + SD]^2), calculated from capillary BGC measurements taken at 5 intraoperative time points. Patients in the intervention group experienced higher CV (16.5% *vs* 10.1%, *P* = 0.008) and J index scores (25.3, range 17.9-39.7 *vs* 18.9, range 16.0-25.3, *P* = 0.046) than the control group. Insulin resistance was calculated using the homeostasis model assessment insulin resistance value (HOMA-IR) = [fasting glucose (mg/dL) × fasting insulin (μU/mL)]/405. Patients in the intervention group experienced higher HOMA-IR scores than the control group (8.5, range 5.6-19.2 *vs* 2.7, range 2.2-4.8, *P* < 0.001). Secondary outcomes included gastric volume, and postoperative complications including nausea, vomiting, dizziness, hypotension, delirium, wound dehiscence, and pain scores. There was no difference between the two groups with respect to gastric volume or any of the reported postoperative complications, except for delirium which was higher in the intervention group (4 *vs* 0, *P* = 0.045)[27].

In summary, several early studies that examined patients without DM demonstrated that PCL significantly improved patient experience (nausea, vomiting, pain) and postoperative muscle function (hand grip strength, peak expiratory flow rate). Administration of a PCL in this patient population also reduced postoperative insulin requirements and improved postoperative BGC. Later studies that did not exclude patients with DM showed that administration of a PCL does not increase the risk for postoperative morbidity in most respects, in particular with regards to aspiration of gastric contents.

DISCUSSION

In this original scoping review, the clinical relevance of GV and the clinically significant relationship between GV and surgical outcomes were described. The available evidence on the impact of PCL on GV and surgical outcomes in patients with and without DM was presented. High GV has clear negative implications in both patients with and without DM in a wide range of inpatient clinical settings; however, it remains uncertain whether PCL reduces GV perioperatively and improves surgical outcomes in this patient population.

The clinical impact of GV has been studied extensively, in particular as a predictor of morbidity and mortality in patients with and without DM in a variety of inpatient clinical settings, including surgical and non-surgical. Several different indices of GV, including SD, CV, GLI, and MAGE, show a correlation with morbidity and mortality, and so practitioners that use this data point may reasonably select whichever index is most accessible for their practice setting. At the same time, the lack of a gold standard GV index may reduce standardization across study designs and produce clinical data that is more challenging to compare. Two studies presented in this scoping review suggest that GLI may be the most accurate predictor[14,15]; however, one study recommends CV as the most practically accessible [20].

There is a lack of consensus on both the carbohydrate composition and the volume of an optimal PCL [28]. The type of dextrose-containing solutions used in the reviewed PCL studies varied. Additionally,

the timing of PCL administration varied throughout the examined literature. Future research to elucidate the optimal type and timing of PCL administration would allow subsequent clinical trials to follow more standardized protocols and therefore more definitively determine the risks and benefits of the PCL.

Of the studies analyzed for this scoping review, there is a paucity of evidence investigating the impact of a PCL on perioperative GV. The one such study included in this review did find an increase in GV after PCL administration in 46 patients with DM; however, the investigators analyzed BGC obtained from capillary blood, which may not be as accurate as whole blood[29]. In a retrospective analysis of 83 non-diabetic patients undergoing colorectal surgery, investigators found that a PCL with complex carbohydrates had a beneficial impact on GV when compared to a PCL with simple carbohydrates[30]. More studies looking directly at the effect of a PCL on GV indices are needed before a consensus determination can be reached. Similarly, there is insufficient evidence to determine that PCL improves surgical outcomes for patients with and without DM, though it does not appear to be associated with worse outcomes.

Despite the widespread exclusion of patients with DM in early PCL studies, there is a significant body of evidence suggesting that PCL is safe in patients with well controlled type 2 DM. A narrative review of emerging evidence on PCL safety and effectiveness in patients with type 2 DM suggested that consuming a PCL raises preoperative BGC; however, the PCL did not significantly impact intraoperative or postoperative BGC[30]. Additionally, the PCL improved patient satisfaction measures postoperatively without increasing the risk for complications such as aspiration of gastric contents, pneumonia, and postoperative surgical site infection[31-34]. Of note, because the PCL reduces GV by stimulating endogenous insulin secretion, it is not recommended for those with insulin deficiency such as type 1 DM and should be used with caution in patients with poorly controlled type 2 DM or severe insulin resistance[35]. Large randomized placebo controlled trials investigating the PCL could ultimately determine whether it improves a variety of clinical outcomes or is solely a non-inferior intervention that improves patients' perioperative comfort and satisfaction.

This scoping review was intended to link clinical concepts together with a historical perspective to identify knowledge gaps and research opportunities pertaining to present day practice. It was designed to summarize emerging evidence pertaining to perioperative GV, PCL, and postoperative outcomes in patients with and without DM. By specifying an aim early on, all relevant literature was collected and gaps in knowledge were identified. This process allowed for recommendations for future research to be made based on where current research is lacking or non-existent.

This scoping review does not incorporate all of the available literature pertaining to this broad topic that may otherwise have been included in a systematic review. Instead, this scoping review encompassed some aspects of glycemic control that are interconnected clinically but may be conceptually separated in literature searches. Each of the three broad topics discussed could be presented as an individual systematic review. A literature search that included all of these elements systematically would be cumbersome.

Given the limitations of a scoping review, there is the possibility that some available evidence has not been mentioned or cited. This is not because the authors have an underlying conflict of interest. None of the authors have any personal interest or conflict of interest with regards to this topic.

CONCLUSION

In conclusion, the benefits of a PCL outweigh the risks in most patients, even those with type 2 DM. The administration of a PCL might effectively minimize metabolic derangements such as GV and ultimately result in reduced postoperative morbidity and mortality, but this remains to be proven. Future efforts to standardize the content and timing of a PCL are needed. Prospective studies should be appropriately designed to evaluate the PCL effect on GV indices in the immediate postoperative period, and on long term postoperative complications in patients with and without DM.

FOOTNOTES

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Diabetes and cognitive decline: Challenges and future direction

Norhamidar Ab-Hamid, Norsuhana Omar, Che Aishah Nazariah Ismail, Idris Long

Norhamidar Ab-Hamid, Idris Long, Biomedicine program, School of Health Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kota Bharu 16150, Kelantan, Malaysia

Norsuhana Omar, Che Aishah Nazariah Ismail, Department of Physiology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kota Bharu 16150, Kelantan, Malaysia

Corresponding author: Idris Long, PhD, Academic Research, Senior Lecturer, Biomedicine program, School of Health Sciences, Health Campus, Universiti Sains Malaysia, Jalan Hospital USM, Kubang Kerian, Kota Bharu 16150, Kelantan, Malaysia. idriskk@usm.my

Abstract

There is growing evidence that diabetes can induce cognitive decline and dementia. It is a slow, progressive cognitive decline that can occur in any age group, but is seen more frequently in older individuals. Symptoms related to cognitive decline are worsened by chronic metabolic syndrome. Animal models are frequently utilized to elucidate the mechanisms of cognitive decline in diabetes and to assess potential drugs for therapy and prevention. This review addresses the common factors and pathophysiology involved in diabetes-related cognitive decline and outlines the various animal models used to study this condition.

Key Words: Diabetes mellitus; Insulin signaling; Macrovascular disease; Microvascular disease; Animal models; Cognitive decline; Pathophysiology

Core Tip: Diabetes can induce cognitive decline, a phenomenon attributed to fluctuations in glycemic status, macrovascular and microvascular disease, deterioration of insulin signaling, neuroinflammation, mitochondrial dysfunction, increases in advanced glycation end products, the effects of drugs used to treat diabetes, and diabetic autonomic dysfunction. Various animal models have been constructed to examine the pathophysiology of diabetes-induced cognitive decline.

INTRODUCTION

According to current data from the International Diabetes Federation (IDF), more than 1 in 10 ten persons has diabetes. Diabetes prevalence among adults (20 to 79 year of age) has more than quadrupled from 151 million (4.6% of the global population) in 2000 to 537 million (10.5% of the global population) in 2021. More shockingly, it is estimated that without changes to intervention strategy, 643 million people (11.3% of the world population) will develop diabetes by 2030[1].

Diabetes complications are primarily attributed to vascular and metabolic factors associated with the disease. Among the major complications are cardiovascular disease, stroke, peripheral artery disease, nephropathy, retinopathy, neuropathy, dental disease, and immunocompromise. These are also expected to become increasingly pervasive, affecting both the local and global burden of illness[2]. Diabetes is a systemic disease, as it can affect nearly every body system. For instance, diabetes can disrupt proper cardiovascular, gastrointestinal, immune, or nervous function. The functional impairment of the peripheral nervous system can lead to diabetic foot and, in the worst cases, amputation and associated physical disability. Diabetic retinopathy can lead to loss of vision and blindness. A wide range of cognitive dysfunction can also occur as a consequence of diabetes, and can manifest as mild cognitive impairment (MCI) to dementia[3].

There is an increased risk of cognitive decline and dementia in patients with diabetes[4]. This has major implications for patient care, particularly in older adults with dementia or pre-dementia with cognitive impairment, which are the most typical manifestations in communities worldwide[4]. The stages of diabetes-associated cognitive decline depend on the type of diabetes and also the patient's age. For type 1 diabetes (T1DM), the impairment of cognitive function progresses with age. However, for type 2 diabetes (T2DM), there are three stages of cognitive function loss, including diabetes-associated cognitive decline, MCI, and in the final stage, dementia[2].

Several investigations have revealed that patients with T1DM may suffer from severe impairments in information processing, psychomotor efficiency, attention, visuoconstruction, and mental flexibility[5]. However, T2DM has been associated more with problems in executive function, psychomotor speed, and memory. As a result, older diabetic patients often have slower walking pace, poorer coordination, a higher chance of falling, and more fractures, all of which can affect quality of life. In addition, executive dysfunction has been linked to the incapacity to carry out daily tasks.

The effects of diabetes on brain function and cognitive decline have received little attention in academia. However, a study using brain magnetic resonance spectroscopy discovered various metabolic criteria for dementia in diabetic patients and established new links between dementia and diabetes. This study also found extremely low levels of N-acetyl aspartate (which affects neuronal integrity), high levels of myoinositol, high levels of excitatory neurotransmitters (*e.g.,* glutamate and glycine), and low levels of inhibitory neurotransmitters [*e.g.,* gamma-aminobutyric acid (GABA)], which has been linked to pain perception problems in diabetic patients[6]. Diabetes also causes brain atrophy, myelin degradation, and vacuole dispersion throughout the white matter of the brain in rats[7]. Diabetic patients are also thought to have irregularities in the metabolism of neurotransmitters in the brain, which leads to neuronal dysfunction and destruction, eventually contributing to the development of dementia.

Research regarding the actions of insulin has mainly focused on peripheral diseases rather than brain function[8]. However, insulin has been shown to play a role in cognition and neuroprotection in brain. Insulin also has an indirect effect on brain function by acting on peripheral tissue. Many circulating mediators that fluctuate due to obesity and diabetes can pass across the blood-brain barrier (BBB) and contribute to dysfunction in neurons, astrocytes, and microglia[7]. Nonetheless, the mechanism of diabetes-induced cognitive decline is still uncertain. Interestingly, this condition shares many cellular and molecular pathways with Alzheimer's disease (AD), the most common form of dementia[9]. Here, we describe the putative pathophysiology of diabetes that may contribute to cognitive decline and review diabetic animal models used to study this condition. Finally, we discuss the obstacles and future directions for elucidating the diabetes-related mechanisms associated with cognitive decline.

FACTORS AND PATHOPHYSIOLOGY OF DIABETES INDUCES A COGNITIVE DECLINE

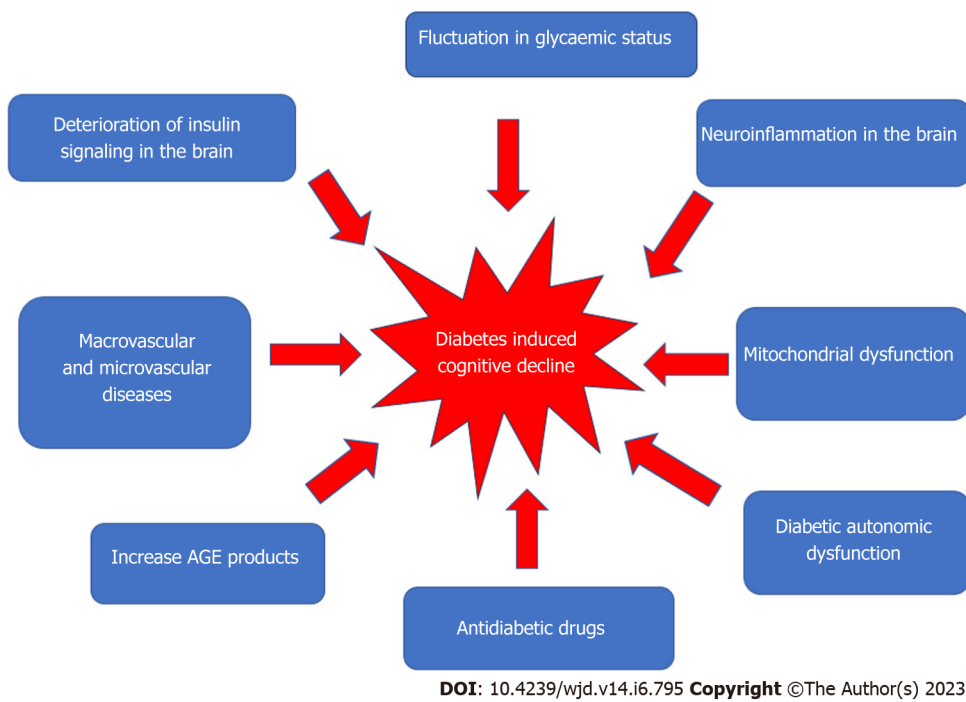Figure 1 shows the factors that may contribute to the development of diabetes-induced cognitive decline.

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Figure 1 Multifactorial pathophysiology of diabetes-induced cognitive decline.

Macrovascular and microvascular diseases

Many vascular, metabolic, and psychosocial factors have been linked to diabetic-induced cognitive decline. Vascular disease, including hypertension and dyslipidemia, has been linked to an increased risk of stroke; in diabetic patients, this risk is estimated to be 115% higher for every 1% increase in glycated hemoglobin (HbA1c)[10]. Furthermore, cardio- or cerebrovascular abnormalities in the brain can lead to cognitive decline and dementia. Patients with T1DM who frequently have cognitive difficulties may have both subclinical and overt cerebrovascular disease[3]. T2DM patients with elevated plasma trigly-cerides and higher cholesterol levels have been demonstrated to have poorer cognitive function[11]. Studies have also revealed a relationship between cognitive dysfunction and hypertension in T2DM[6, 7]. However, due to inconsistent findings from observational studies in the general population[8,9], the roles of dyslipidemia and hypertension in the development of cognitive decline in diabetics is still uncertain and needs further investigation.

Microvascular dysfunction also has been associated with cognitive decline[3,12]. Chronic hyperglycemia increases the risk of microvascular dysfunction, which can affect many organs, including the eye (retinopathy), kidney (nephropathy), and nerves (neuropathy). There is also a positive correlation between the development of cognitive decline and the presence of nephropathy and/or retinopathy[13]. Retinopathy has been linked to cognitive decline in adult diabetic patients as it is thought to affect intelligence, attention/concentration, and information processing[14].

Hyperglycemia

Hyperglycemia has been linked to cognitive decline[15], and can affect cognitive function in the long and short term time. Hyperglycemia has been shown to correlate with impaired working memory, attention, and depression. Acute variations in blood glucose have a negative effect on cognitive performance, and glycemic control improvement is advantageous for regulating cognitive function. It does not cause microvascular structural changes, but has been linked to regional cerebral blood flow or osmotic shifts across the neuronal membrane[16]. In contrast, chronic hyperglycemia affects cognitive function through the production of advanced glycosylation end-products (AGEs), formation of senile plaques and neurofibrillary tangles, and cerebral microvascular disease[17]. A reduction in white matter volume has also been connected to diminished executive function and a reduction in the processing of information[18].

The negative impact of hyperglycemia on cognitive impairment was validated in a zebrafish study in which T1DM was induced with injection of streptozotocin (STZ)[19]. It was discovered that exposing zebrafish to water-diluted glucose for 14 d caused sustained memory impairment accompanied by an increase in acetylcholinesterase activity. On the other hand, galantamine therapy reversed the memory-damaging effects of hyperglycemia. These findings revealed a link between acetylcholinesterase activity and cognitive impairment in T1DM patients[19].

However, cross-sectional studies investigating the association of chronic hyperglycemia (as evidenced by HbA1c) and cognitive decline in people with T2DM have yielded inconclusive results[20, 21]. However, this association is apparent in older patients, as the improvement in glycemic control also improves cognitive function[22]. One study demonstrated that treatment of T2DM with either rosiglitazone or glibenclamide (glyburide) improved working memory over 24 wk[16]. Metformin has also been shown to reduce the risk of cognitive impairment in diabetic patients[23], but other evidence suggests that it may increase the risk[24] or have no effect[25]. Treatment with metformin may reduce tau phosphorylation as well as interleukin-1ß-mediated activation of the phosphokinases Akt and mitogen-activated protein kinase (MAPK). Furthermore, it can inhibit the mitochondrial respiratory chain, increasing cyclic adenosine monophosphate (AMP) and activating protein kinase A and AMP-activated protein kinase (AMPK)[26]. AMPK activation has been shown to improve memory and learning in female animal models[27]. However, when the evidence from observational studies and randomized controlled trials is combined, it seems that hyperglycemia and glucose excursions are both weakly associated with poorer cognitive function in T2DM patients[28]. As a result, further research is needed regarding hyperglycemia as a potentially modifiable risk factor for cognitive decline in diabetes.

Hypoglycemia
The presence of hypoglycemic episodes in diabetic patients has also been linked to cognitive decline and an increased risk of dementia[17,18]. The human brain, which accounts for 20% of the body's metabolic consumption, has a greater need for glucose as a fuel source than other parts of the body. As a result, if the brain is temporarily depleted of glucose, cognitive and emotional functions are impaired. If left untreated, neuroglycopenia can lead to coma, seizure, or brain damage. There is evidence that repeated severe hypoglycemia in patients with early-onset diabetes can contribute to slower mental development and lower intellectual quotient (IQ)[29]. While the cerebral effects of severe hypoglycemia in adults are still not fully clear, insulin-dependent diabetic adults with repeated episodes of severe hypoglycemia performed worse on neuropsychological tests than diabetic patients who had never experienced severe hypoglycemia[30,31]. Another study found a weak link between the reported frequency of severe hypoglycemia and IQ decrement, lower levels of current IQ, and slowed variable reaction times[32]. However, this study found no cognitive differences between diabetic patients receiving intensive insulin therapy with severe hypoglycemia and those receiving conventional therapy[33]. The impact of several episodes of severe hypoglycemia between the ages of 5 year and 15 year is considered mild among young adults dependent on insulin. Strict glycemic control is thought to have a significant benefit in reducing target organ damage and slowing the progression of nephropathy, retinopathy, and neuropathy; however, it increases the risk of severe hypoglycemia. Further research on hypoglycemia and cognitive decline is needed to assist diabetic patients and their physicians in making the best treatment decisions.

Hyperinsulinemia
Hyperinsulinemia caused by endogenous insulin hypersecretion is common in the early stages of T2DM as a result of insulin resistance (IR). Hyperinsulinemia in adults without diabetes is associated with poorer cognitive function[26-28] and an increased risk of AD[34]. When compared to normal patients, patients with moderate to severe AD had higher levels of insulin in plasma but lower levels in cerebrospinal fluid[35]. Insulin therapy, both intravenous and intranasal, has been shown to improve cognitive function in AD patients[36]. Insulin injection into the cerebral ventricles of rats has also been shown to improve memory in a study that demonstrated an insulin signaling defect similar to that found in peripheral tissues could also occur in the hippocampus, resulting in functional insulin deficiency and cognitive decline[37]. Rosiglitazone can prevent disruption in memory tasks and reduce β-amyloid protein in the brain in transgenic mice that overexpress human amyloid precursor protein and develop AD pathology[38,39]. However, more research is needed to determine the link between hyperinsulinemia and cognitive decline.

Peripheral and cerebral insulin resistance
Insulin is released into the circulation by the pancreas and can pass the BBB *via* a carrier-facilitated process. The BBB comprises ependymal and endothelial cells, and the blood-cerebral spinal fluid barrier has insulin-binding sites that allow insulin to pass through[7]. Insulin receptors are found in the hypothalamus, prefrontal cortex, and hippocampus, among other central nervous system (CNS) sites [9]. The activation of hippocampal insulin receptors is thought to mediate insulin-induced cognitive improvement in healthy mammalian brains by facilitating long-term hippocampal potential (LTP), which is linked to learning and memory, as well as by increasing the expression of N-methyl-D-aspartate (NMDA) receptor[10] (Figure 2). Insulin also regulates the production of other neurotransmitters involved in learning and memory, including acetylcholine, norepinephrine, and adrenaline[3], and stimulates the accumulation of GABA-A receptors on the postsynaptic membrane[11]. A transient surge in peripheral insulin is thought to cause an increase in CNS insulin, which reaches the brain.

In healthy individuals, insulin binds to insulin receptor α-subunits and stimulates the tyrosine kinase domain of βsubunits, resulting in autophosphorylation. This autophosphorylation has the potential to

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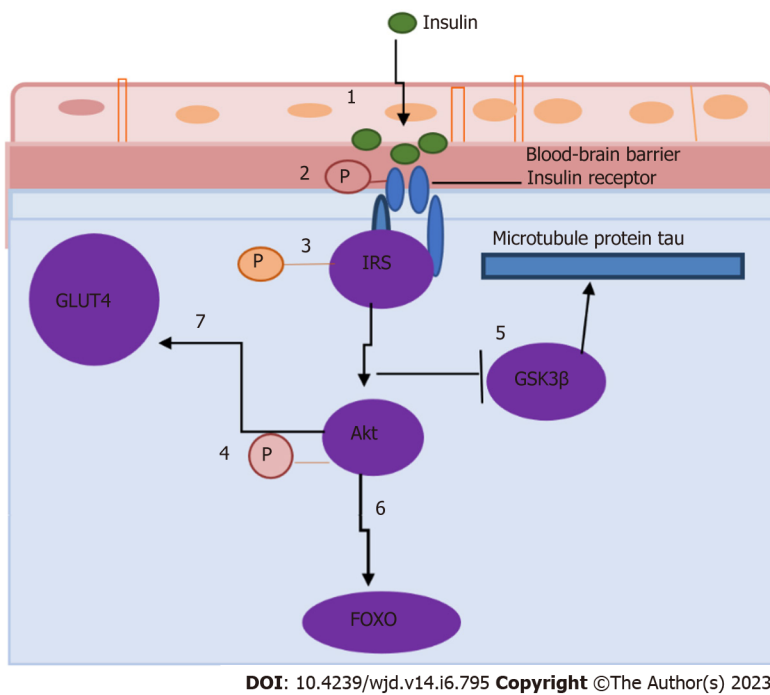
Figure 2 Insulin receptor signaling in the hippocampus (adapted from Biessels and Reagan[40], 2015). Cerebral insulin resistance causes downregulation of insulin transporters at the blood-brain barrier, limiting the amount of insulin that can enter the brain (step 1), decreasing the expression and/or activity of insulin receptors (step 2), modulating the phosphorylation state of insulin receptor substrates (step 3), phosphorylation of Akt (step 4), affecting several downstream components in the insulin signaling cascade (including glycogen synthase kinase 3β) (step 5), regulating the phosphorylation state of the microtubule protein tau and forkhead box O family of transcription factors (step 6), and impairing the trafficking of GLUT4 to the plasma membrane of the brain (step 7). GSK3β: Glycogen synthase kinase 3β; FOXO: Forkhead box O.

activate the phosphoinositide 3-kinase (PI3K)-Akt (also known as PKB) signaling pathway. Akt molecules (Akt1, Akt2, and Akt3) are serine/threonine kinases that are activated by PI3K in response to growth factors and other cellular stimuli. In the brain, Akt mediates the translocation of glucose transporter type 4 (GLUT 4; also known as SLC2A4) to the plasma membrane. Akt also phosphorylates and inactivates the forkhead box O (FOXO) transcription factor family and glycogen synthase kinase 3β (GSK3β), reducing GSK3β's ability to phosphorylate the microtubule-associated protein tau.

Chronic peripheral hyperinsulinemia induced by diabetes, obesity, or hyperlipidemia produces peripheral IR associated with the brain's functional and structural changes. It also contributes to the dysregulation of insulin signaling in the brain and the development of cerebral IR. Cerebral IR causes the downregulation of insulin transporters at the BBB, limiting the amount of insulin that can enter the brain, decreasing the expression and/or activity of insulin receptors, and modulating the phosphorylation state of insulin receptor substrates such as Akt[7]. T2DM patients have lower Akt activation in their adipocytes and skeletal muscle, leading to many damaging effects on neuronal and glial cells[40]. Lower Akt activation affects several downstream components in the insulin signaling cascade, including GSK3β, which regulates the phosphorylation state of the microtubule protein tau and FOXO family of transcription factors. As a result, trafficking of GLUT4 to the plasma membrane is impaired. In addition, memory problems, diminished neuroprotective effects, and impaired synaptic transmission may result from cerebral IR, all of which may also contribute to the development of neurodegenerative illness[20]. However, the mechanisms underlying the relationships between systemic metabolic and vascular consequences of peripheral IR and cerebral IR are still largely undefined. Observational studies in humans are unlikely to fully elucidate the complex interplay between local and systemic factors of IR in the brain and periphery with respect to the mechanism of diabetes-induced cognitive decline[41,42].

Mitochondrial dysfunction

Mitochondria are involved in oxidative respiration, energy metabolism, free radical production, and apoptosis among other physiological processes[43]. The brain has a high energy requirement, and as such it is particularly sensitive to mitochondrial dysfunction. Mitochondria play an important role in anti-aging and neurodegenerative disease prevention[44]. The pathogenesis of diabetes and many neurodegenerative diseases includes mitochondrial dysfunction, attributed to the production of reactive oxygen species (ROS) that can damage proteins, carbohydrates, and lipids. Dysfunctional mitochondria are less effective in generating ATP but rather produce more ROS, leading to the oxidative imbalance seen in cognitive decline[45].

One study has reported that hyperglycemia in diabetes enhances mitochondrial oxidative stress and ROS generation, which can lead to calcium homeostasis disruption, apoptosis, and memory impairment [45]. Diabetic rats also had higher levels of superoxide, protein oxidation, and thiobarbituric acid reactive substances[46] as well as reduced activities of catalase, superoxide dismutase, and glutathione peroxidase in the brain[47]. Excessive oxidative stress causes release of cytochrome C, which starts the apoptotic cascade and leads to mitochondrial dysfunction[44]. These findings suggest that diabetes may worsen mitochondrial dysfunction and oxidative stress in memory and cognition-related brain regions, and may be the fundamental cause of diabetes-related cognitive decline.

Neuroinflammation in the Brain

Diabetes raises the levels of pro-inflammatory cytokines in the brain, which can lead to neuronal damage[48]. Additionally, vascular endothelial dysfunction also elevates inflammatory mediators and compromises the BBB. When BBB function is impaired, neurotoxic blood proteins such as thrombin, fibrin, plasmin, and hemoglobin can potentially enter the brain parenchyma, causing abnormal neuronal activity[49]. The pro-inflammatory nuclear factor-kappa B (NF-κB) has been implicated in diabetic cognitive decline, and a pharmacological inhibitor that inhibits NF-κB activation has been shown to reduce levels of interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) and improve cognitive decline[50].

A post-mortem examination of a diabetic patient's hippocampus revealed microglia activity similar to that seen in AD patients[17]. TNF-α levels and microglial activation in the brain were found to be higher in mice fed a high-fat diet. It has also been suggested that diabetes and obesity cause decreased spatial recognition memory in db/db mice, which is linked to increased levels of pro-inflammatory cytokines, establishing a relationship between inflammation and cognitive loss[18]. In addition, there is a relationship between neuroinflammation and ROS in cognitive impairment. The creation of ROS in the diabetic brain has been shown to stimulate several cellular pathways, including the advanced glycation end products and its receptor (AGE/RAGE), polyol, and protein kinase C pathways, leading to increased brain inflammation and neurodegeneration[49].

Increase in AGEs

Hyperglycemia in diabetes damages tissues and increases intracellular glucose. This condition triggers mitochondrial overproduction of reactive oxygen and nitrogen species (RONS) such as superoxide anion radical, peroxynitrite, and hydrogen peroxide[51]. RONS, in turn, cause DNA damage and overstimulate peroxisome proliferator-activated receptor, a repair enzyme that increases NAD consumption while decreasing the activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which is already compromised by RONS[52]. As a result, endothelial dysregulation occurs, as does the initiation of pro-apoptotic signals, such as the production of AGEs. When AGEs interact with specific receptors (RAGEs), a complex pro-inflammatory cascade involving IL-1, IL-6, TNF-α, transforming growth factor-β (TGF-β), and vascular cell adhesion molecule-1 (VCAM-1) is activated, increasing oxidative stress[51-53]. AGE formation alters the structural and functional properties of proteins in both the extracellular matrix and the intracellular region.

Effects of drugs used in the treatment of diabetes

When compared to untreated patients, treated diabetic patients have less improperly aggregated protein and less vascular damage[54,55]. Metformin, an insulin sensitizer, can reduce the risk of dementia and the rate of cognitive decline in diabetics[54]. Biguanides and sulfonylureas, among other diabetes medications, can alter the relationship between tau pathology and diabetes, slowing the onset of cognitive decline[55].

It is postulated that the best way to delay the onset of dementia is to improve early prevention strategies. Both elderly and middle-aged people with diabetes have poorer cognitive functioning and faster cognitive deterioration[56]. A retrospective study found that the association between DM and AD is stronger in middle-aged people than in the elderly, implying that age is a significant factor in the relationship between DM and AD.

Diabetic autonomic dysfunction and cognitive impairment

Diabetes autonomic dysfunction (DAD) is a complication of diabetes with unexplained and undiscovered pathogenesis. DAD is related to poor blood pressure regulation and a higher risk of stroke, both of which are risk factors for cognitive impairment[57]. Cognitive decline and autonomic dysfunction have comparable fundamental pathologic mechanisms. Autonomic function is compromised in patients with MCI, AD, frontotemporal dementia, dementia with Lewy bodies, and Parkinson's disease with dementia. In comparison to age-matched controls with normal cognition, there is evidence of sympathetic cardiac autonomic dysfunction in patients with MCI[30]. The correlation between blood pressure dysregulation, silent cerebral infarcts, and cognitive decline reveals that intermittent chronic declines in cerebral blood flow caused by high blood pressure can lead to cognitive decline. This process, however, is still not fully clear and requires additional investigation.

ANIMAL MODELS OF DIABETES-INDUCED COGNITIVE DECLINE

Zucker diabetic fatty rat

Zucker diabetic fatty (ZDF) rats are a genetically derived Zucker fatty strain model of pathological alterations associated with T2DM. Obese ZDF rats have T2DM symptoms such as increased insulin levels, obesity, and increased triglyceride levels. Genetically, the ZDF rat model has also been reported to have abnormally low brain insulin content[58]. Studies have found that this model has altered memory tests and hippocampal-dependent learning due to hyperinsulinemia[59,60]. It is believed that leptin receptor deficiency in the hippocampus of ZDF rats impairs LTP in the hippocampal CA1 region and affects spatial memory[61]. Another study found that the brains of ZDF rats produced more ROS and nitric oxide, as well as suffered more complications of redox homeostasis, mitochondrial function, and ATP synthesis[62]. Astrogliosis was discovered in the hippocampus and frontal and parietal cortices, and there was an increase in the number of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes in ZDF rats[63]. ZDF rats also exhibit reduced hypothalamic corticotropin releasing factor tone due to dysregulation of the hypothalamic-pituitary-adrenal axis[64].

The db/db mouse

The db/db mice are now being used to generate diabetes in rodents to better understand the underlying mechanism and etiology of T2DM. This model includes a leptin receptor gene mutation that causes hepatic IR, hyperglycemia, hyperinsulinemia, hyperlipidemia, and obesity[24]. The Morris water-maze (MWM) test reveals impaired spatial memory in these mice due to decreases in their leptin receptors in the hippocampus[61]. The interaction between cytokines and central processes involving the hippocampus contributes to cognitive behavioral alteration in this db/db mice[65]. Reportedly, changes in hippocampal plasticity and function in db/db mice can be reversed when normal physiological levels of corticosterone are maintained, indicating that cognitive impairment in this model may be caused by glucocorticoid-mediated deficits in neurogenesis and synaptic plasticity[66].

The released cytokines, such as IL-1, due to obesity and diabetes in this model can also mediate the neuroinflammation process and impair hippocampal synaptic plasticity[66]. The debilitation of memory and learning process in db/db mice due to metabolic changes has the ability to reduced membrane metabolism and tricarboxylic cycle and also restrain the cycle of Gln-Glu/GABA, impartially triggering a rise in anaerobic glycolysis[67]. Similarly, Yermakov *et al*[68], who observed a study on the db/db model in the MWM test's reversal phase, confirmed that it would impact cognitive flexibility. Another study using this model was executed to examine the importance of neutrophils in the db/db mice model after exposure to hypoxic/ischemic (H/I) insults, which might generate higher morbidity and acute ischemic stroke[69].

The ob/ob mouse

Ob/ob mice are a naturally occurring genetic model in which a mutation in the leptin gene causes leptin insufficiency. As a result, they have large appetites, develop obesity, and are considered an appropriate model for T2DM. A study has been done in ob/ob mice to identify the effect of T2DM disease on tau phosphorylation, which concluded that tau hyperphosphorylation affected thermoregulation resulting in hypothermia in ob/ob mice[68]. The ob/ob mice with leptin-deficiency showed an increase in LTP in the amygdala, indicating that diabetes can have an impact on emotional state[70]. A previous study on ob/ob mice showed acute behavioral dysfunction and disability of spatial memory with higher pro-inflammatory cytokine levels and NF-κB activation compared to the control[71]. Another study looked at the lifespan of the ob/ob mouse and found a link between this and the dysregulation of microglia and astrocytes. Higher levels of GFAP and decreased levels of microglial markers followed this finding[72].

Goto-Kakizaki rat

The Goto-Kakizaki (GK) rat was developed from a polygenic non-obese Wistar substrain as a non-obese diabetic animal model for spontaneous T2DM. A study of brain energy metabolism in diabetic GK rats using 13C magnetic resonance spectroscopy found that the glutamate-glutamine cycle between astrocytes and neurons was impaired due to astrocytes having a greater TCA cycle rate than neurons [73]. Soares *et al*[74] (2019) also demonstrated that diminished brain glycogen metabolism could interfere with memory and learning capability in the GK rat model. The present findings resulted in the successful induction of aging as one of the characteristics of AD in advanced-age GK rats by increasing phosphorylation of tau. Furthermore, there was an increase in amyloid-β levels along with a reduction in the levels of synaptic proteins in GK rats[75].

High-fat diet rats and streptozotocin injection

In comparison to the regular diet of rats, a high-fat diet (HFD) represents a diet with a high-fat content mixed with fructose or glucose. One study showed that C57Bl/6 mice fed a high-fat lard diet increased their body weight and had impaired cognitive function due to increased brain inflammation and decreased BDNF levels[76]. Rats fed high-calorie diets such as HFD, high glucose, and high fructose diets demonstrated changes in energy and lipid metabolism similar to those seen in clinical diabetes,

including elevated blood glucose, cholesterol, and triglycerides. This high-calorie diet also decreased spatial learning ability, hippocampal dendritic spine density, and LTP at Schaffer collateral-CA1 synapses. These changes occurred in tandem with a decrease in BDNF levels in the hippocampus[77-79]. This effect has also been proposed due to increased corticosterone and peripheral IR, which may contribute to cerebral IR and increase oxidative stress reaction in the brain[80,81].

Many studies have found that rats fed HFD paired with low-dose STZ also developed obesity and cerebral IR, two key hallmarks of T2DM[82,83]. The T2DM rat model closely resembled the natural history of disease events to induce IR, impair β cell malfunction and metabolic characteristics of T2DM. STZ is an anti-neoplastic and antibiotic drug isolated initially from *Streptomyces achromogenes* in 1960 and consists of a nitrosourea moiety that is interposed between a methyl group and glucosamine. Due to its severe toxicity to mammalian pancreatic β cells, this drug is commonly used in research to generate experimental animal models of T1DM and AD. Its diabetogenic effects are manifested as hypoinsulinemia, hyperglycemia, polydipsia, and polyurea in animals, all of which are characteristic features of diabetes in humans. Although high-dose STZ causes severe impairment in insulin secretion comparable to that seen in T1DM, low-dose STZ has been shown to cause a modest impairment in insulin secretion, which is similar to T2DM in its later stages[83]. This model is easily available, cheaper, and valuable for future research.

CHALLENGES AND FUTURE DIRECTION

The role of insulin in the brain, particularly the hippocampal region, has been demonstrated to be critical for functional and structural changes in the brain for cognitive processes. Insulin plays a trophic role in the brain and serves as a metabolic homeostasis regulator, promoting neuroplasticity and high energy regulation. Understanding the molecular mechanisms of insulin on brain plasticity is critical for identifying the mechanisms that regulate neural plasticity in health and metabolic disease, such as diabetes-induced cognitive decline, as well as in neurodegenerative disease, particularly AD[80].

To date, research has confirmed the hypothesis that boosting hippocampal insulin receptor signaling could reverse or ameliorate IR-induced neuroplasticity deficits in animal models of T2DM[40]. Previous research has also shown that pharmacological and lifestyle interventions can effectively restore hippocampal neuroplasticity in a T2DM animal model[81]. Several studies study also looking into the efficacy of intranasal insulin administration as an innovative therapeutic strategy to alleviate cognitive decline in T2DM, as it allows insulin to be delivered directly to the CNS and avoids systemic hormone effects[36,84]. Nonetheless, the findings of these studies raise important questions about the localization and effects of intervention strategies, whether they are mediated peripherally or centrally.

The diabetic animal model, which has been used to replicate human cognitive decline, has some limitations and is unreliable in determining the exact human brain condition in diabetes. In addition, diabetes-related cognitive decline has a convoluted etiology with several variables, such as IR and insulin insufficiency, as well as pancreatic cell malfunction, all of which can lead to multiorgan deficits. Thus, additional new characteristics of animal models, along with clinical evidence, should be empowered.

As in T1DM, the induction of STZ is involved in pharmacologic toxicity by destroying pancreatic β cells, which is carcinogenic[85]. The challenge of the STZ-induced animal model involves higher mortality of rats due to toxicity is a stumbling block in research. As the toxicity of STZ can impact multiple organs, it can resemble a contribution to death instead of diabetic complications[86].

Regarding the development of T2DM animal models, potential systemic consequences of disrupted leptin signaling in ob/ob mice to exhibit diabetic peripheral neuropathy should be contemplated[87]. Ob/ob and db/mice are assigned as an appropriate model for neuropathy diabetes, exhibiting early onset and approximate nature of neuropathy. However, numerous studies have shown that these models can result in infertility. Furthermore, they could not perpetuate hyperglycemia levels that are inconsistent with the reduction in fasting blood glucose started at the age of 4 wk[88].

HFD rat models can be developed for future investigations that imitate other human conditions. Nevertheless, the diet composition may not work well with interstudy data. To better understand disease pathogenesis and therapeutic approaches by employing animal models, standardization of induction methods and extensive phenotyping should be prioritized.

Furthermore, the HFD and STZ injection models are more expensive and require a long time to develop. However, animal experimental models that carry significant heterogeneity of diabetes pathology across a broad spectrum of phenotypes seen in patients with cognitive decline must be developed and improved in order to make progress in investigating the causative mechanisms of cognitive decline in diabetes, particularly in T2DM.

CONCLUSION

The pathophysiology of diabetes-induced cognitive decline is still elusive. The proposed molecular mechanisms are derived from fluctuation in glycemic status that led to macrovascular and microvascular dysfunction in blood vessels, an increase in AGEs that trigger cerebral IR in the brain, the occurrence of neuroinflammation, and mitochondrial dysfunction that activates apoptosis. Drugs used to treat diabetes also may contribute to diabetes-associated cognitive decline. Furthermore, diabetic autonomic dysfunction can also be linked to cognitive decline, but the mechanism is still unknown. The pathophysiology of diabetes-induced cognitive decline may have a similar mechanism to AD, which includes development of IR in the brain, especially in hippocampus region; IR has been shown to affect neuroplasticity during cognitive processing. Further studies and the creation of reliable animal models to fully understand how diabetes causes cognitive decline are needed. Understanding the association between diabetes and cognitive decline will provide a better understanding of pathogenesis and cognitive decline in diabetic patients, which may assist future researchers in developing potential interventions to alleviate the resulting symptoms of this disease.

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Effect of resveratrol in gestational diabetes mellitus and its complications

Hui-Zhong Ma, Yuan Chen, Hao-Hao Guo, Jing Wang, Xiu-Lan Xin, Yan-Cheng Li, Yu-Feng Liu

Hui-Zhong Ma, Yuan Chen, Hao-Hao Guo, Jing Wang, School of Pharmaceutical Sciences, Liaoning University, Shenyang 110036, Liaoning Province, China

Xiu-Lan Xin, College of Bioengineering, Beijing Polytechnic, Beijing 100176, China

Yan-Cheng Li, Department of Epidemiology, University of Florida, Gainesville, FL 32608, United States

Yu-Feng Liu, School of Pharmaceutical Sciences, Natural Products Pharmaceutical Engineering Technology Research Center of Liaoning Province, Liaoning University, Shenyang 110036, Liaoning Province, China

Corresponding author: Yu-Feng Liu, PhD, Professor, School of Pharmaceutical Sciences, Natural Products Pharmaceutical Engineering Technology Research Center of Liaoning Province, Liaoning University, No. 66 Chongshan Mid-Road, Huanggu District, Shenyang 110036, Liaoning Province, China. liuyufeng@bjmu.edu.cn

Abstract

The incidence rate of diabetes in pregnancy is about 20%, and diabetes in pregnancy will have a long-term impact on the metabolic health of mothers and their offspring. Mothers may have elevated blood glucose, which may lead to blood pressure disease, kidney disease, decreased resistance and secondary infection during pregnancy. The offspring may suffer from abnormal embryonic development, intrauterine growth restriction, obesity, autism, and other adverse consequences. Resveratrol (RSV) is a natural polyphenol compound, which is found in more than 70 plant species and their products, such as *Polygonum cuspidatum*, seeds of grapes, peanuts, blueberries, bilberries, and cranberries. Previous studies have shown that RSV has a potential beneficial effect on complex pregnancy, including improving the indicators of diabetes and pregnancy diabetes syndrome. This article has reviewed the molecular targets and signaling pathways of RSV, including AMP-activated protein kinase, mitogen-activated protein kinases, silent information regulator sirtuin 1, miR-23a-3p, reactive oxygen species, potassium channels and CX3C chemokine ligand 1, and the effect of RSV on gestational diabetes mellitus (GDM) and its complications. RSV improves the indicators of GDM by improving glucose metabolism and insulin tolerance, regulating blood lipids and plasma adipokines, and modulating embryonic oxidative stress and apoptosis. Furthermore, RSV can ameliorate the GDM complications by reducing oxidative stress, reducing the effects on placentation, reducing the adverse effects on embryonic development, reducing

offspring's healthy risk, and so on. Thus, this review is of great significance for providing more options and possibilities for further research on medication of gestational diabetes.

Core Tip: Resveratrol (RSV) is a natural polyphenol compound. Previous studies have shown that RSV has a potential beneficial effect in complex pregnancy, including improving the indicators of diabetes and improving pregnancy diabetes syndrome. This article reviews the molecular targets and signaling pathways of RSV including AMP-activated protein kinase, mitogen-activated protein kinases, silent information regulator sirtuin 1, miR-23a-3p, reactive oxygen species, potassium channels and CX3C chemokine ligand 1, and the effect of RSV on gestational diabetes mellitus and its complications. It also provides more options and possibilities for further research on medication of gestational diabetes.

INTRODUCTION

Diabetes is a metabolic disease caused by islet dysfunction, insulin resistance (IR), and other factors. Its clinical manifestation is hyperglycemia. Among them, type 1 diabetes refers to the inability of the body to produce enough insulin, and type 2 diabetes refers to the inability of cells to respond appropriately to insulin. Another type of diabetes is called gestational diabetes mellitus (GDM), which occurs when the blood glucose level of pregnant women is high.

Approximately 20% of all pregnancies are complicated by GDM, which includes hyperglycemia, IR, and fetal maldevelopment. Several factors contribute to the development of GDM, including low-grade inflammation in the mother and peripheral IR. Sterile inflammation and infection are key mediators of this inflammation and IR[1,2]. Due to the severe complications it causes to both mother and fetus, GDM is a serious problem worldwide[3]. At present, insulin and hypoglycemic western medicine are mainly used in clinical treatment. Pregnant B safe drugs and insulin treatment are mainly selected according to the blood glucose situation. However, long-term use of insulin will do harm to mothers and fetuses. Therefore, actively exploring natural non-toxic phytochemicals to prevent and treat diabetes during pregnancy is a future development trend.

Based on many *in vitro* and animal studies, dietary polyphenols have been shown to inhibit hyperglycemia, IR, inflammatory adipokines, and modify microRNA profile *via* the insulin signaling pathway[3]. Since the early 1990s, polyphenols have been extensively studied as adjuvant agents to attenuate obesity, cardiovascular disease, malignancies, neurodegenerative diseases, diabetes, and metabolic syndrome. Resveratrol (RSV) is one of the most studied natural polyphenols, with health benefits clearly demonstrated in various *in vitro* and *in vivo* models, as well as in clinical studies[4].

RSV belongs to the stilbene-type phytophenol, it is found in more than 70 plant species and their products such as *Polygonum cuspidatum*, seeds of grapes, peanuts, blueberries, bilberries, and cranberries [5]. The trans-RSV form, which is the most organic form, and the cis-RSV form are the two forms of RSV (Figure 1). Accumulating evidence suggests that RSV is a biological modulator and phytoalexin with multi-target and multi-action characteristics. In a variety of animal and human models, RSV has exhibited a diverse range of biological effects including cardioprotective[6], anti-hypertensive[7,8], antiobesogenic[9,10], antiatherosclerotic[11-13], potent anti-inflammatory[14], and antidiabetic[15,16] effects.

This article summarizes the mechanism and effect of RSV on GDM and its complications.

PATHWAY AND TARGETS OF RSV IN GDM

There have been many previous studies on the signaling mechanisms involved in diabetes, but there is less reported on RSV signaling pathways and targets in GDM. Studies have confirmed the link between molecular targets and signaling pathways of RSV including AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK), silent information regulator sirtuin 1 (SIRT1), miR-23a-3p,
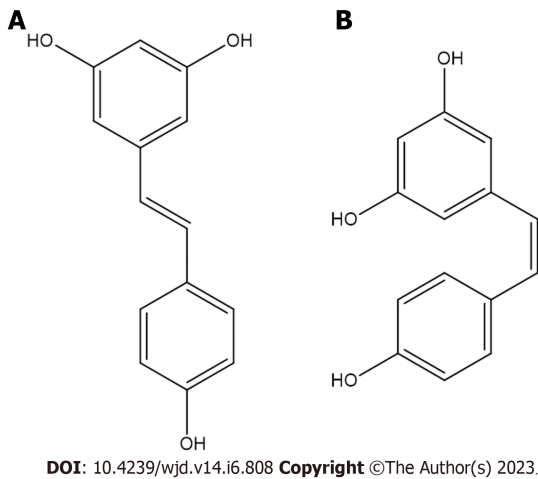
Figure 1 Chemical structures of trans-resveratrol (3,5,4'-trihydroxystilbene) and cis-resveratrol. A: Trans-resveratrol; B: Cis-resveratrol.

reactive oxygen species (ROS), potassium (K) channels, and CX3C chemokine ligand 1 (CX3CL1) (Figure 2).

AMPK

AMPK, a serine/threonine kinase, is conserved in eukaryotes. Under stressful circumstances, AMPK controls cellular and overall body energy homeostasis. It is well established that AMPK dysregulation is associated with a wide range of diseases including cancer[17], diabetes[18], inflammatory illness[19], hypertension, and kidney disease[20], and cardiovascular disease[21]. For optimal placental differentiation, nutrition transport, maternal and fetal energy homeostasis, and membrane protection during pregnancy, AMPK is required[22]. Metformin, RSV, and 5-aminoimidazole-4-carboxamide ribonucleotide are AMPK activators that have been shown to reverse pregnancy problems such as GDM, preeclampsia, intrauterine growth restriction (IUGR), and premature birth in preclinical studies[23].

A previous study investigated inflammation, oxidative stress, apoptosis, and AMPK in embryos on embryonic day 16 in a streptozotocin (STZ)-induced gestational diabetes mouse model. RSV inhibited AMPK activity and expression, which further decreased expression levels of p65, IkappaB kinase beta, and IkappaB alpha. RSV (8.0 mg/kg) administration significantly downregulated expression levels of ROS, superoxide dismutase (SOD), glutathione, and catalase in oxidative stress, and also inhibited inflammatory factors expression such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, C-reactive protein, and IL-6. Mechanism analyses indicated that RSV inhibited inflammation of embryonic cells by the AMPK-mediated nuclear factor kappa B signaling pathway[24].

MAPKs

In diabetes embryos exhibiting developmental abnormalities, a study identified downregulation of retinoid X receptors, retinoic acid receptor (RAR) expression, DNA-binding capabilities, and phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, but an increase of phosphorylation of p38 and c-Jun N-terminal kinase (JNK) 1/2. MAPKs and RARs were activated in rat embryos on embryonic day 12 after treatment with RSV (100 mg/kg body weight), then they displayed normalized patterns of p38, JNK, ERK, and RAR phosphorylation. This finding suggested that RSV might be able to prevent RAR and MAPK dysfunction in the embryos of a mouse model of diabetic embryopathy[25].

SIRTs

SIRTs are involved in metabolic and circulatory processes. Adipocyte differentiation and insulin signaling, which are controlled by forkhead box protein O1 and phosphoinositide 3-kinase (PI3K) signaling, both depending on SIRT1. The path mechanisms of the nonalcoholic hepatitis, cardiovascular illnesses, diabetes mellitus type 2, and metabolic syndrome are partially explained by the decreased expression of SIRTs[26].

In fetal endothelial colony-forming cells (ECFCs) and human umbilical vein endothelial cells (HUVECs) from pregnancies complicated by GDM, the influence of GDM on SIRT expression and activity was researched in a study. RSV significantly increased SIRT expression and activity in HUVECs and ECFCs, which may provide new therapeutic targets in the future[27,28].

Another study's objective was to determine how oxidative stress affected the glucose transporters (GLUTs) and human placenta's glucose absorption. The reduction in GLUT1 expression and glucose uptake caused by hypoxanthine/xanthine oxidase was eliminated in the presence of the SIRT1 activator RSV. The information given here shows that oxidative stress decreases GLUT1 expression and placental glucose absorption through a SIRT1-dependent mechanism[29]. A study of RSV's effects on myocardial
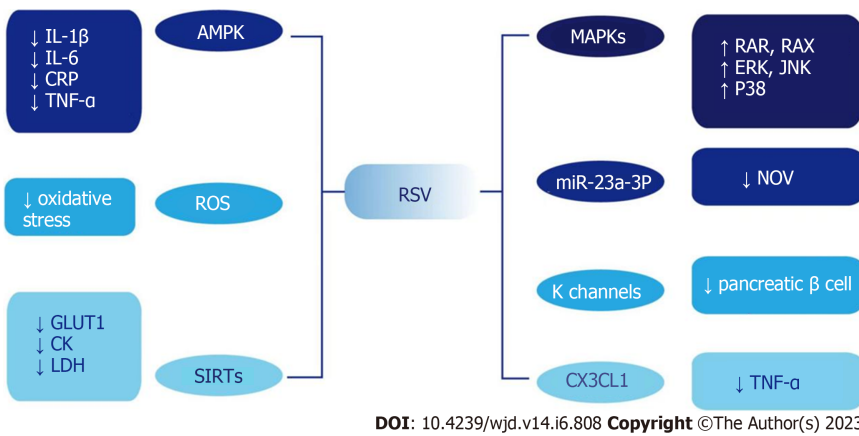
Figure 2 Pathway and targets of resveratrol in gestational diabetes mellitus. AMPK: AMP-activated protein kinase; CK: Creatine kinase; CRP: C-reactive protein; CX3CL1: CX3C chemokine ligand 1; ERK: Extracellular signal-regulated kinase; GLUT1: Glucose transporter type 1; IL: Interleukin; JNK: C-Jun N-terminal kinase; LDH: Lactate dehydrogenase; MAPK: Mitogen-activated protein kinase; NOV: Nephroblastoma overexpressed; RAR: Retinoic acid receptor; RAX: RNA-dependent protein kinase-associated protein X; ROS: Reactive oxygen species; SIRT: Sirtuin; TNF-α: Tumor necrosis factor-alpha.

cell injury also showed the role of the macrophage stimulating 1/SIRT3 signaling pathway in autophagy in type 2 diabetic mice by reducing the body weight of db/db mice, blood glucose level, serum creatine kinase, and lactate dehydrogenase levels[30].

MiR-23a-3p

The low miR-23a-3p expression in diabetic patients controls adipocytes' IR to insulin. Therefore, researchers hypothesized that the effect of RSV on mice with GDM was achieved by controlling miR-23a-3p. Increasing the expression of phosphorylated Akt (p-Akt), miR-23a-3p, p-PI3K, adiponectin, leptin, and glucose intake, as well as decreasing the expression of nephroblastoma overexpressed (NOV) in IR adipocytes were the end results of this study's additional treatment with RSV. This study shows that RSV can improve lipid metabolism and glucose uptake of mice with GDM and IR adipocytes by mediating the miR-23a-3p/NOV axis[31].

ROS

ROS are crucial components of cellular signal transduction and transcriptional regulation, but too much ROS production can damage proteins, cellular lipids, and nucleic acids by oxidative alteration. Indeed, higher levels of ROS are linked to complications induced by diabetes[32].

Transient hyperglycemia produces persistent ROS formation with decreased SOD2 expression, according to the findings of an *in vitro* study. Additionally, *in vivo* rat studies have demonstrated that maternal hyperglycemia causes amygdala SOD2 reduction, which results in autistic-like behavior in offspring. We came to the conclusion that hyperglycemia-mediated chronic oxidative stress and SOD2 reduction caused by maternal diabetes cause autism-like behavior[33].

RSV showed the unusual potential to lower oxidative stress by two separate pathways in both rats and non-human primates since it crosses the placenta in both species. First, improving fetal oxygen delivery and increasing uterine arterial blood flow by working through endothelial nitric oxide synthase. Consequently, reducing ROS generated by hypoxia prevents oxidative damage. Second, to control the genes involved in the redox system directly in fetal tissues. RSV stands out as a potential therapy to utilize as an intervention during a pregnancy complicated by GDM because of these special features[34].

K channels

K channels are essential for sustaining membrane potential. Pathologies include diabetes mellitus, preeclampsia, premature delivery, hypertension, cardiac arrhythmia, and different cancers can all be caused by abnormal K channel activity or expression. K channels may be possible targets in the mechanism of RSV action, according to an article that discusses the pharmacological effects of RSV on the various types of K channels that have been identified in smooth muscle cells[35].

A significant aspect of the pathophysiology of diabetes is the apoptosis of pancreatic beta cells. A study found that the expression of sulfonylurea receptor 1, the regulatory subunit of pancreatic ATP-sensitive K(+) channels, is necessary for RSV to cause beta-cell death[36].

CX3CL1

CX3CL1 contains three exons, is encoded on the long arm of human chromosome 16 at position13. The human placenta exhibits CX3CL1 hyperactivity that is induced by hyperglycemia. RSV has anti-inflam-

matory and antioxidant properties that are influenced by the signaling pathways of the chemokine CX3CL1 and its receptor, CX3CR1. RSV (50 μm and 100 μm) administration into the perfusion fluid decreased TNF-α and CX3CL1 production[37].

INDICATOR IMPROVEMENT OF GDM

We found that administration of RSV works *via* three different ways in GDM: (1) Improving glucose metabolism and insulin tolerance; (2) regulating blood lipids and plasma adipokines; and (3) modulating embryonic oxidative stress and apoptosis[38]. RSV, a potent antioxidant and free radical scavenger, can improve the activities of various antioxidative enzymes and reduce the increasing of ROS, and then reduce the probability of complications caused by diabetes.

Improving glucose metabolism

In a study, RSV significantly enhanced the pregnant db/+ GDM mouse model's insulin tolerance, glucose metabolism, and reproductive outcome (db/+ is a C57BL/KsJ-Lep mouse, which is genetic GDM model that closely resembled human GDM symptoms). Additionally, the researchers discovered that RSV reduced the symptoms of GDM by boosting AMPK activation, which in turn decreased glucose-6-phosphatase expression and activity in both pregnant db/+ females and their offspring[2]. This research provides more evidence in favor of the potential therapeutic benefits of RSV for GDM.

RSV enhanced insulin secretion and restored normoglycemia, glucose tolerance in pregnant dams. At 15 wk of age, the obesity of the male progeny of GDM + RSV-hemifacial spasm (HFS) was lower than that of the offspring of GDM-HFS. Therefore, supplementation of maternal RSV during the third trimester of pregnancy and lactation resulted in a number of positive metabolic health outcomes for mothers and offspring[39]. RSV may be a better option than the GDM therapies now available.

Furthermore, a pilot study found that supplementing with trans-RSV and Revifast in addition to plus D-chiro-inositol/Myo-inositol improves glucose levels, total cholesterol, low-density lipoprotein (LDL), and triglyceride (TG) in overweight pregnant women[40]. This data show that RSV is effective not only for pregnant diabetes mice but also for pregnant humans.

Regulating blood lipids and plasma adipokines

The level of insulin was substantially higher in the RSV treatment group than in the GDM group; however, both the body weight and blood glucose level were markedly decreased. The RSV (240 mg/kg) therapy group had lower levels of LDL cholesterol, TG, total cholesterol (TC) and leptin levels, and higher levels of high-density lipoprotein cholesterol, IL-6, TNF-α, and resistin than the control group. Adiponectin levels were markedly raised and significantly decreased in the 240 mg/kg RSV treatment group. RSV (240 mg/kg) was also more effective than metformin hydrochloride at regulating adipokine levels, controlling blood cholesterol levels, and increasing insulin secretion. RSV lowered blood glucose and body weight, increased insulin secretion, and controlled plasma adipokines and blood lipids in GDM rats in a dose-dependent manner[41].

A study found that maternal RSV therapy reduced the increase in leptin/soluble leptin receptor ratio caused by maternal high-fat (HF) exposure during pregnancy and changed the expression levels of genes for essential fatty acid manufacturing enzymes in the offspring. Thus, to lessen the harmful effects of GDM, maternal RSV administration may be employed[42].

In a study of human mature adipocytes, after the fat cells were incubated with 100 μM RSV (45 min to 4 h), RSV increased in triacylglycerol decomposition induced by isoproterenol stimulation, and showed an impairment of insulin antilipolytic action, after which the production of fat was significantly impaired[43].

Modulating embryonic oxidative stress and apoptosis

According to a study, RSV may be a useful treatment option for women who are pregnant with diabetes because it can improve glucose and insulin levels, improve glucose and lipid metabolism, prevent apoptosis, and reduce inflammation and embryonic oxidative stress in mice with GDM[24].

In ob/ob mice given RSV, plasma levels of insulin and testosterone levels, whereas the homeostatic index of resistance increased. After RSV therapy in obese mice, TNF-α and IL-6 levels returned to nearly normal levels. RSV administration led to considerably more oocytes being harvested in wild-type mice [44].

Coating chitosan with RSV bioactive compounds is an important way for the management of GDM. The treatment of RSV-zinc oxide complex coated chitosan (CS-ZnO-RS) maintained the lipid content and dramatically reduced the blood glucose concentrations of GDM induced rats. Additionally, the levels of inflammation-related components [monocyte chemoattractant protein-1 (MCP-1) and IL-6] as well as endoplasmic reticulum stress (p-PERK, p-eIF2α, p-IRE1α, and GRP78) were decreased by CS-ZnO-RS[45].

In a study, RSV treatment significantly improved defects in the glucose uptake and insulin signaling pathway caused by lipopolysaccharide, TNF-α, and poly (I:C) and significantly decreased the secretion and expression of pro-inflammatory cytokines IL-1β, IL-6, IL-1α, and pro-inflammatory chemokines MCP-1 and IL-8 in omental, human placenta, and subcutaneous adipose tissue. Taken together, these findings indicated that RSV lowered IR and inflammation generated by chemical and microbial agents, and RSV might be a helpful prophylactic treatment for pregnancies complicated by IR and inflammation [46].

AMELIORATION OF GDM COMPLICATIONS

Although pregnancy causes IR condition, it is natural and aids in the provision of glucose to the fetus's circulation and diffusion-mediated transfer of glucose into the placenta[47]. Multiple pregnancy complications can occur if blood glucose concentrations are not properly managed, and this suboptimal in utero environment is likely to affect fetal growth at critical developmental windows. Important organ systems undergo harmful structural changes in utero that last into adulthood and put offspring at a higher risk of developing non-communicable chronic metabolic disorders like obesity, diabetes and cardiovascular disease[34].

Many bioactive redox modulators are used during pregnancy; for example, vitamin C and vitamin E supplements can reduce the risk of pre-eclampsia[48], maternal treatment with a mitochondria-targeted antioxidant can provide protection during hypoxic pregnancy[49], and lazaroid (lipid peroxidation inhibitor) administered along with a low protein diet prevents blood pressure elevation[50]. Also, maternal supplementation with RSV has been used as a therapeutic agent for pregnancy complications in rodent models such as preeclampsia[51], GDM, and fetal growth restriction[52]. It has been reported that the safe dose of RSV for humans is 5 g per day[53]. Relevant studies about RSV intake and the effect are summarized in Table 1.

Reducing oxidative stress

RSV may act directly on diabetic pregnant embryos through normalizing oxidative stress induced by hyperglycemia[54]. Apoptosis induced by oxidative stress is related to diabetic embryopathies[55]. In embryos, RSV can modulate oxidative stress marker normalization, including increases in total thiol concentrations, lipid peroxidation and decreased amounts of glutathione associated with hyperglycemia. The weakening of oxidative stress further decreased and reduced the chance of apoptosis as well as embryonic malformations[38].

RSV was discovered to stop oxidative stress and apoptosis in developing embryos. In a rodent model of diabetic embryopathy, RSV (100 mg/kg body weight) administration improved lipid (triglyceride 60.64%, cholesterol 41.74%), and the glucose (33.32%) profile of the diabetic dams, demonstrating the protective effect of RSV on diabetic pregnancy[54]. Therefore, RSV's antioxidant capability is a desirable property for reducing oxidative stress during challenging pregnancies and thereby breaking the intergenerational cycle of chronic disease.

Using STZ at a dose of 50 mg/kg to cause diabetes in pregnant rats on day 4, followed by 100 mg/kg of RSV on days 8 to 12 to promote neurulation. Fetuses were taken on the 19th day of pregnancy and submitted to morphologic investigation. The activities of the glutathione peroxidase, superoxide dismutase, and scavenging enzymes catalase in the fetal liver were also assessed. RSV has been demonstrated with embryo protective effects that are mediated by reducing the oxidative stress brought on by maternal hyperglycemia[56].

By administering 100 μM tert-butylhydroperoxide (tert-BOOH) for 24 h, oxidative stress was created in a human syncytiotrophoblast (STB) cell model, the BeWo cell line. The reduced STB glucose buildup was accompanied by an increase in transepithelial permeability. The inhibitory effect of tert-BOOH on 2-cleoxyglucose was fully reversed by RSV thanked to a particular effect on transport mediated by glucose transporters[57]. This result demonstrated that RSV may influence the results of pregnancy disorders linked to oxidative stress.

Reducing adverse effects on placentation

The key players in placentation, an early process essential for placental growth and function that involves an appropriate invasion and through remodeling of the maternal spiral arteries during early pregnancy, are extravillous trophoblasts (EVTs). The finding of a study indicated that oxidative stress interferes with EVT features necessary for the placentation process, which may help explain the link between pregnancy disorders and oxidative stress[58].

Using a first trimester extravillous human trophoblast cell line (HTR8/SVneo cells) as a cell model, our goal was to examine the impact of high levels of leptin, insulin, TNF-α, and glucose (indicators of diabetes in pregnancy), on the process of placentation. Therefore, insulin may have an impact on placentation[59]. Because placental formation was affected by leptin and insulin, so we can use RSV to regulate insulin release, thus protecting the placenta.

Table 1 Relevant studies about resveratrol intake and the effect

Model	Species	Resveratrol consumption	Duration of treatment	Maternal outcomes	Offspring outcomes	Ref.
C57BL/6	Mice	8.0 mg/kg	16 gestation days	Inhibit expression levels of inflammatory factors, IL-1β, IL-6, CRP and TNF-α↓		[24]
C57BL/KsJ-, Lepdb/+ (db/+)	Mice	10 mg/kg	During pregnancy	Glucose metabolism, insulin tolerance↑, glucose-6-phosphatase↓		[2]
C57BL/6	Mice	0.20%	18 gestation days	p-Akt, miR-23a-3p, p-PI3K, adiponectin, leptin↑		[31]
Female ob/ob mice, female C57BL/6J mice	Mice	3.75 mg/kg	20 d	Plasma insulin and T levels↓, IL-6, TNF-α levels reverted back to normalcy		[44]
Female Sprague-Dawley rats	Rats	100 mg/kg	10 gestation days		p38, JNK, ERK, and RAR phosphorylation return normal	[25]
Female Sprague-Dawley rats	Rats	240 mg/kg	12 gestation days	TC, TG, LDL-C, leptin, resistin, TNF-α, and IL-6↓, HDL-C, adiponectin↑		[41]
Female Sprague-Dawley rats	Rats	147 mg/kg	3-wk lactation period	Blood glucose levels↓, insulin secretion↑	Male offspring obese↓, hepatic steatosis, insulin resistance, glucose intolerance and dysregulated gluconeogenesis↓	[39]
Pregnant rats	Rats	100 mg/kg	10 gestation days	Glucose and lipid profile↑	Embryo weight↑, rump length, somite number↓	[54]
Pregnant rats	Rats	100 mg/kg	4 d (gestation days 8th to 12th)	-	Teratogenic effects↓, scavenging enzymes catalase, superoxide dismutase, glutathione peroxidase↓	[56]
Hypoxia-induced rat model of IUGR	Rats	4 g/kg	9 wk		Intra-abdominal fat deposition, accumulation of TG and ceramides↓, plasma lipid profile↑	[62]
Chicken embryo	Chicken	1 nM/egg	5 embryonic days		Death rate, developmental damage, vessel injury↓	[61]
Between the 24th and 28th weeks' gestation	Human	80 mg/day	60 d	Total cholesterol, HDL, LDL, triglycerides, and glucose blood levels↓		[40]
Mature adipocytes	Human	100 μM	45 min to 4 h	Isoprenaline stimulation↑, impaired insulin antili-polytic action		[43]
Placenta, omental and subcutaneous adipose tissue and skeletal muscle	Human	200 μM	20 h	IL-6, IL-1α, IL-1β, pro-inflammatory chemokines IL-8, MCP-1↓		[46]
Heparinized placentae	Human	50 and 100 μM, 5 mL boluses at 30 min intervals	150 min		CX3CL1, TNF-α↓	[37]

CRP: C-reactive protein; CX3CL1: CX3C chemokine ligand 1; ERK: Extracellular signal-regulated kinase; HDL: High-density lipoprotein; IL: Interleukin; IUGR: Intrauterine growth restriction; JNK: C-Jun N-terminal kinase; LDL: Low-density lipoprotein; MCP-1: Monocyte chemoattractant protein-1; RAR: Retinoic acid receptor; TC: Total cholesterol; TG: Triglyceride; TNF-α: Tumor necrosis factor-alpha.

Reducing adverse effects on embryonic development

Given that organogenesis and embryonal development are the most delicate stage of development, it is recognized that diabetes may impair these processes. Potential cause of the observed embryonal deformity in diabetic dams is oxidative stress induced by hyperglycemia, which results in apoptosis[4]. Numerous complications might arise during a diabetic pregnancy, particularly about embryo development. Inadequate or incomplete closure of the neural tube, impaired rate of neurogenesis, developmental delay, and failure to generate the right neural connections are a few examples of embryonic impairments[60]. Furthermore, several upcoming neurological, physical, and psychiatric illnesses may have their roots in these developmental problems. Diabetic malformations are more likely to happen in the first trimester.

In this work, the impact of RSV on the development of chicken embryos in conditions of high glucose and the RSV's underlying mechanism were examined. At the embryonic day 1, the high glucose concentration to chicken embryos caused growth retardation, stillbirth, and poor yolk sac blood vessel development. RSV supplementation had a substantial impact on reducing developmental harm, mortality, and vascular injury before glucose exposure. Aside from that, exposure to high glucose levels resulted in oxidative stress, which RSV might treat. Furthermore, excessive glucose dramatically reduced the neuronal developmental marker paired box 3, which was thereafter restored by RSV. RSV also interfered with gene expression that is controlled by the cell cycle. This study discovered a link between hyperglycemia-induced embryonic damage and RSV, which raised the possibility of RSV having a protective impact[61].

Reducing offspring's healthy risk

A study showed that RSV administration improved the plasma lipid profile, decreased intra-abdominal fat deposition, and reduced accumulation of TG and ceramides in the tissues of offspring with IUGR. Additionally, RSV reduced glucose intolerance and IR, decreased Akt signaling in the skeletal muscle and liver of offspring with IUGR, and activated AMP-activated protein kinase, all of which may have led to better metabolic parameters in IUGR rats treated with RSV. The findings implied that early postnatal RSV treatment could enhance the metabolic profile of HF-fed infants born from IUGR-complicated pregnancies[62].

DISCUSSION

Compared with synthetic drugs, RSV may become a safer and more effective natural drug to treat or prevent pregnancy diabetes and its complications. However, due to the low bioavailability and water solubility of RSV (< 0.05 mg/mL), we can start from two aspects: Modifying its structure to find derivatives with higher activity or developing new dosage forms through new carriers. At present, various RSV derivatives have been widely studied, including methoxylated, hydroxylated and halogenated derivatives[63,64]. In the preparation research, chitosan has been used to encapsulate CS-ZnO-RSV[45], a new RSV nano delivery system based on lipid nanoparticles[65], galactosylated poly lactic-co-glycolic acid nanoparticles for the oral delivery of RSV[66], and the microparticulate system for delivering liquid and solid microparticles of RSV[67]. These research bases provide the goal and direction for continue study of RSV absorption in depth.

In addition, RSV could reduce steroidogenesis in rat ovarian theca-interstitial cells by inhibiting of Akt/protein kinase B signaling pathway[68], and might enhance normal-weight females' responses to controlled ovarian hyperstimulation by RSV's anti-inflammatory, insulin-sensitizing, and antihyperandrogenism mechanisms[36]. Also there were evidences showed that RSV attenuated lipid peroxidation, sperm DNA damage[69] and alleviated testicular cell apoptosis in type 1 diabetes mice [70]. These observations demonstrated RSV's therapeutic potential for preserving ovarian reserve and male sperm quality, we can further increase the research on the beneficial effects of RSV on female and male reproduction, and believe that it is a good direction for the research of RSV.

CONCLUSION

As a natural polyphenol compound, RSV has the advantages of low side effects, wide sources, low price, and low safety risk. RSV could improve the indicators of GDM by improving glucose metabolism and insulin tolerance, regulating blood lipids and plasma adipokines, and modulating embryonic oxidative stress and apoptosis. Furthermore, RSV could ameliorate the GDM complications by reducing oxidative stress, reducing the effects on placentation, reducing the adverse effects on embryonic development, reducing offspring's healthy risk and so on. RSV has high application value in pregnancy diabetes and its complications, some of its targets are directly affected, while others are modulated indirectly, through changes in their expression levels. This may not only be the advantage of RSV in treating diabetes, but also may bring some unexpected side effects. Therefore, in order to meet the needs of users, it is still necessary to conduct in-depth research on RSV in the process of use.

FOOTNOTES

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Basic Study

Comprehensive analysis of endoplasmic reticulum stress-related mechanisms in type 2 diabetes mellitus

Bo Liang, Shu-Wen Chen, Yuan-Yuan Li, Shun-Xiao Zhang, Yan Zhang

Bo Liang, Department of Nephrology, The Key Laboratory for the Prevention and Treatment of Chronic Kidney Disease of Chongqing, Chongqing Clinical Research Center of Kidney and Urology Diseases, Xinqiao Hospital, Army Medical University (Third Military Medical University), Chongqing 400037, China

Shu-Wen Chen, Yuan-Yuan Li, Shun-Xiao Zhang, Yan Zhang, Department of Endocrinology, Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai 200000, China

Corresponding author: Yan Zhang, MD, PhD, Academic Editor, Professor, Department of Endocrinology, Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine, No. 181 Youyi Road, Baoshan District, Shanghai 200000, China. yan_2999bs@163.com

Abstract

BACKGROUND
The endoplasmic reticulum (ER) is closely related to a wide range of cellular functions and is a key component to maintain and restore metabolic health. Type 2 diabetes mellitus (T2DM) is a serious threat to human health, but the ER stress (ERS)-related mechanisms in T2DM have not been fully elucidated.

AIM
To identify potential ERS-related mechanisms and crucial biomarkers in T2DM.

METHODS
We conducted gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) in myoblast and myotube form GSE166502, and obtained the differentially expressed genes (DEGs). After intersecting with ERS-related genes, we obtained ERS-related DEGs. Finally, functional analyses, immune infiltration, and several networks were established.

RESULTS
Through GSEA and GSVA, we identified several metabolic and immune-related pathways. We obtained 227 ERS-related DEGs and constructed several important networks that help to understand the mechanisms and treatment of T2DM. Finally, memory CD4^{+} T cells accounted for the largest proportion of immune cells.

CONCLUSION

This study revealed ERS-related mechanisms in T2DM, which might contribute to new ideas and insights into the mechanisms and treatment of T2DM.

Key Words: Endoplasmic reticulum stress; Type 2 diabetes mellitus; Biomarkers; Memory CD4+ T cells

Core Tip: This study revealed endoplasmic reticulum stress-related mechanisms in type 2 diabetes mellitus (T2DM), which might contribute to new ideas and insights for the mechanisms and treatment of T2DM.

INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces[1,2]. Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the heart, blood vessels, eyes, kidneys, and nerves[3,4]. Recently, the estimated prevalence of diabetes among children, adolescents, and adults has increased[5,6]. The majority of people with diabetes have type 2 diabetes mellitus (T2DM)[7]. Simple lifestyle measures have been shown to be effective in preventing or delaying the onset of T2DM[8]. Recently, with the in-depth understanding of the mechanisms of T2DM, many new drugs, such as sodium-glucose cotransporter-2 inhibitors, glucagon-like peptide-1 analogs, and dipeptidyl peptidase-4 inhibitors, have been gradually applied to clinical practice and achieved good results[9-11]. However, the residual risk of these populations remains high, especially when combined with other diseases[12].

The endoplasmic reticulum (ER) is closely related to a wide range of cellular functions and is a key component to maintain and restore metabolic health[13]. Protein handling, modification, and folding in the ER are tightly regulated processes that determine cell function, fate, and survival[14]. Many genetic and environmental damages hinder the ability of cells to correctly fold and post-translationally modify secreted and transmembrane proteins in the ER, resulting in the accumulation of misfolded proteins in this organelle, which is called ER stress (ERS)[15]. Chronic ERS is becoming a key factor in more human diseases, including T2DM[16,17]. Recently, the biological mechanisms of ERS in T2DM have been gradually explored. *YIPF5* mutations can disrupt the ER-to-Golgi trafficking, thereby resulting in T2DM [16]. Inositol-requiring enzyme 1alpha upregulates miR-200a degradation and stimulates TXINP/ NLRP3-pathway-mediated pyroptosis and renal damage in T2DM[18]. Mfn2 plays an important role in ERS, and Mfn2 silencing prevents mitochondrial Ca2+ overload-mediated mitochondrial dysfunction [19]. ATF5 is a regulator of ERS and β-cell apoptosis in different models of diabetes mellitus[20]. Lactogens modulate the ERS pathway, causing enhanced β-cell survival and reduced T2DM incidence [21]. The development of ERS for the treatment of T2DM has also emerged in clinical trials. A randomized placebo-controlled crossover trial indicated that decreased ERS may lead to improvement of insulin sensitivity mediated by hyperbaric oxygen[22]. Nevertheless, the role of ERS in T2DM, especially the related markers and mechanisms, is still lacking.

Here, we conducted gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) in both proliferating myoblasts and differentiated myotubes, which are important in T2DM. Then, the differentially expressed genes (DEGs) and ERS-related DEGs between T2DM patients and healthy populations were investigated, sequentially. Furthermore, functional enrichment analysis [Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG), immune infiltration analysis, and three networks [transcription factor (TF)–mRNA, miRNA–mRNA, and drug–mRNA] were detected to explore the mechanisms and potential therapeutic agents of ERS in T2DM. The flow chart is shown in Figure 1.
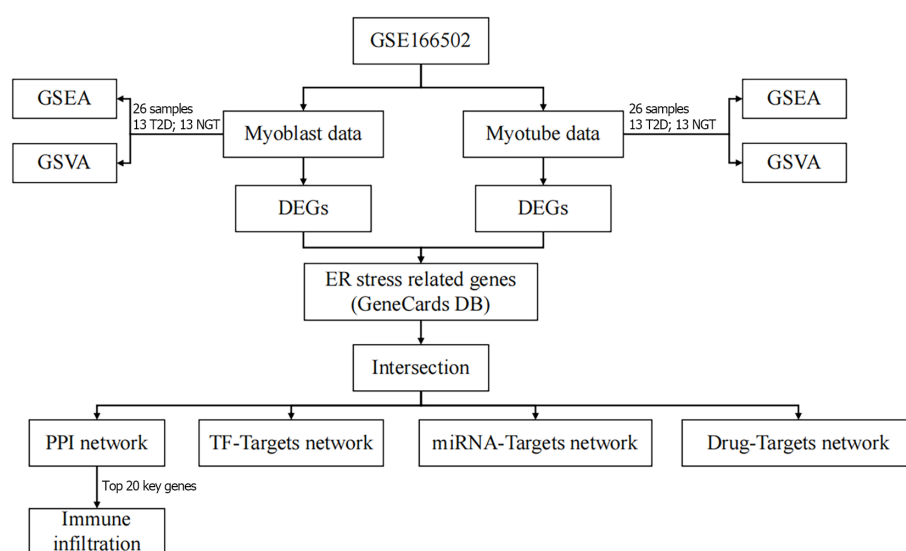
DOI: 10.4239/wjd.v14.i6.820 **Copyright** ©The Author(s) 2023.

Figure 1 Study flow chart. GSEA: Gene set enrichment analysis; GSVA: Gene set variation analysis; ER: Endoplasmic reticulum; DEG: Differentially expressed genes; PPI: Protein–protein interaction; TF: Transcription factor.

MATERIALS AND METHODS

Acquisition and processing of raw data

The raw data of the microarray expression dataset GSE166502[23] and its annotation file GPL10558 (Illumina HumanHT-12 V4.0 Expression BeadChip) were obtained from Gene Expression Omnibus[24]. GSE166502 holds the mRNA expression in proliferating myoblasts and differentiated myotubes in patients with T2DM (n = 13) or controls (n = 13).

GSEA and GSVA

We selected and downloaded c2.cp.v7.2.symbols.gmt gene set data through the GSEA database[25], and conducted GSEA on the proliferating myoblasts and differentiated myotubes through the *clusterProfiler* package (version 3.14.3)[26]. The statistical process of GSEA was to calculate the enrichment score, estimate the significance, and correct the multiple hypothesis tests. We also selected the same data from GSEA and conducted GSVA. The different pathways were obtained through the *limma* package (version 3.42.2)[27].

Identification of DEGs

After the processing of raw data, we analyzed the data using the *limma* package with a fold change and P for DEGs. The threshold of DEGs was |log₂fold change| > 0.263 and P < 0.05 as described previously, and the results were visualized as a heat map and volcano map using the *pheatmap* package (version 1.0.12).

Acquisition of ERS-related DEGs

GeneCards provides annotated and predicted human gene information, which integrates gene data from about 150 network sources, including genomics, transcriptomics, proteomics, genetics, and clinical and functional information[28]. In this study, ERS-related genes were downloaded through GeneCards with "endoplasmic reticulum stress" as the search keyword. Taking the intersection of DEGs and ERS-elated genes, we got the ERS-related DEGs and the Venn diagram was drawn through the *Venndiagram* package (version 1.6.20).

Functional enrichment analysis

GO and KEGG pathway analysis can contribute to the interpretation of system-level data and enable discoveries[29,30]. In this work, GO terms and KEGG analysis of ERS-related DEGs and potential molecular complex were carried out using the *clusterProfiler* package with P < 0.05, and then visualized by the *ggplot2* package (version 3.3.3), as described previously[31].

Protein–protein interaction analysis

Protein–protein interaction (PPI) is one of the cores of cellular processing. The analysis of PPI makes the relationships among proteins clear and helps the function explanation of potential protein complexes or functional modules. In this work, PPI information was surveyed using the String database (version 11.0)

[32]. The PPI network of ERS-related DEGs was uploaded to Cytoscape (version 3.8.2)[33] and the NetworkAnalyzer plugin was used to further processing and analysis. The cytoHubba plugin was used to select the top 20 key genes[34].

Network analysis

Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST, version 2) manually curated database of human and mouse transcriptional regulatory networks[35]. Current TRRUST contains 8444 and 6552 TF-target regulatory relationships of 800 human and 828 mouse TFs. TRRUST database also provides information on the mode of regulation (activation or repression). miRWalk (version 3.0) stores predicted data obtained with a machine-learning algorithm including experimentally verified miRNA–target interactions[36]. The drug–gene interaction database (DGIdb, version 4.2.0) builds drug–gene interactions mined from DrugBank, PharmGKB, Chembl, Drug Target Commons, Therapeutic Target Database, and others[37]. DGIdb contains > 40000 genes and > 10000 drugs involved in > 100000 drug–gene interactions or belonging to one of 42 potentially druggable gene categories. We obtained the TFs, miRNAs, and drugs of ERS-related DGEs, respectively, and then constructed the regulation relationship networks through Cytoscape.

Correlation analysis of immune infiltration

CIBERSORT (version 1.03) calculates the proportion of different types of cells according to LM22[38]. The proportion of different cell types can be calculated after the nonnegative matrix decomposition of the expression matrix. In this study, the immune infiltration of GSE166502 was analyzed by CIBERSORT, and the infiltration of 22 kinds of immune cells in the sample was analyzed. Finally, we analyzed the correlation between the expression of the top 20 key genes in the PPI network and the immune infiltration.

RESULTS

GSEA and GSVA

Through GSEA, we found that neuroactive ligand–receptor interaction, hypertrophic cardiomyopathy, DNA replication, cell cycle, and cardiac muscle contraction were the top five pathways in proliferating myoblasts (Figure 2A–F). DNA replication, cell cycle, cardiac muscle contraction, neuroactive ligand–receptor interaction, and hypertrophic cardiomyopathy were activated, whereas glycosaminoglycan biosynthesis heparan sulfate, glycosaminoglycan biosynthesis chondroitin sulfate, glycosaminoglycan degradation, other glycan degradation, and lysosome were suppressed (Figure 2G). Other pathways, such as arachidonic acid metabolism, mismatch repair, P53 signaling pathway, metabolism of xenobiotics by cytochrome P450, and prion diseases, were also enriched (Figure 2H). Similarly, we found that viral myocarditis, steroid hormone biosynthesis, hematopoietic cell lineage, focal adhesion, and extracellular matrix (ECM) receptor interaction were the top five pathways in differentiated myotubes (Figure 2I–N). Neuroactive ligand–receptor interaction, gap junction, pathways in cancer, focal adhesion, and ECM receptor interaction were activated, whereas steroid hormone biosynthesis, cardiac muscle contraction, viral myocarditis, hematopoietic cell lineage, and steroid biosynthesis were suppressed (Figure 2O). Vascular endothelial growth factor (VEGF) signaling pathway, cell adhesion molecules cams, mitogen-activated protein kinase (MAPK) signaling pathway, and apoptosis were also enriched (Figure 2P).

Through GSVA, eight pathways were enriched in proliferating myoblasts (Figure 3A and B). RNA degradation, DNA replication, and mismatch repair were upregulated, and glycosaminoglycan biosynthesis chondroitin sulfate, other glycan degradation, lysosome, glycosaminoglycan biosynthesis heparan sulfate, and steroid biosynthesis were downregulated. Two pathways (steroid hormone biosynthesis and steroid biosynthesis) were enriched in differentiated myotubes (Figure 3C and D).

Identification of ERS-related DEGs

We performed DEG analysis on proliferating myoblasts and differentiated myotubes. We obtained 426 DEGs (188 upregulated and 238 downregulated, Figure 4A and B) and 281 DEGs (135 upregulated and 146 downregulated, Figure 4C and D) from proliferating myoblasts and differentiated myotubes, respectively. Through intersecting with 6893 ERS-related genes, we obtained 227 ERS-related DEGs (Figure 4E).
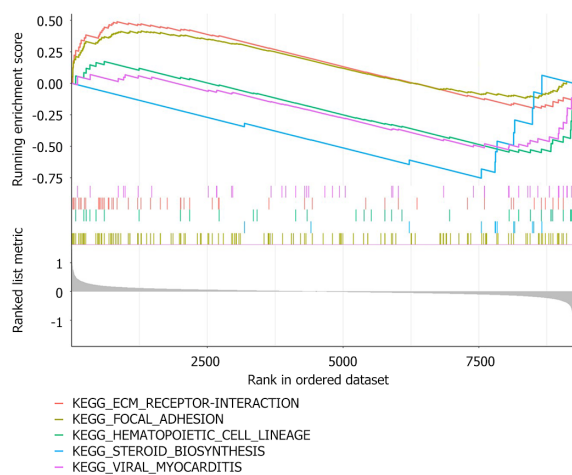
Function enrichment analysis

GO terms include biological processes, molecular functions, and cellular components. There were 227 ERS-related DEGs enriched in 875 biological process terms, 103 molecular function terms, and 81 cellular component terms. The results indicated that numerous biological processes were involved in extracellular structure organization, collagen fibril organization, ECM organization, cellular response to external stimulus, response to ketone, cellular response to fatty acid, cellular response to prostaglandin
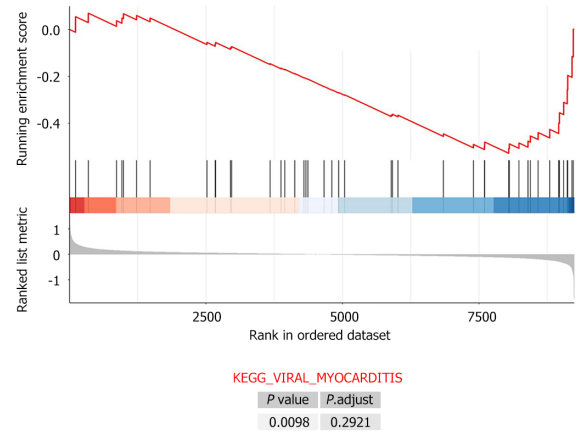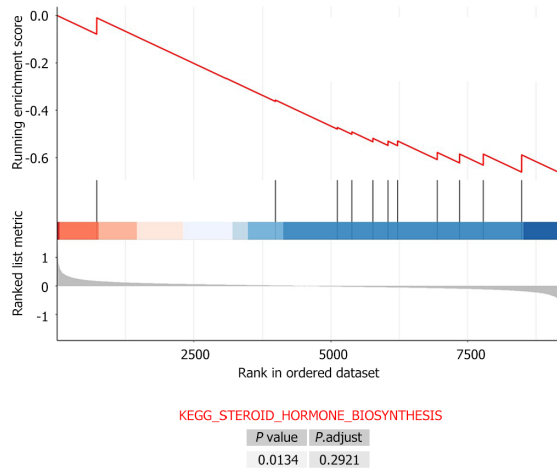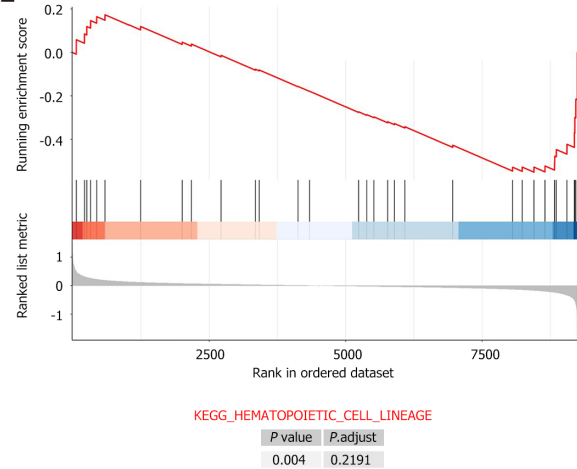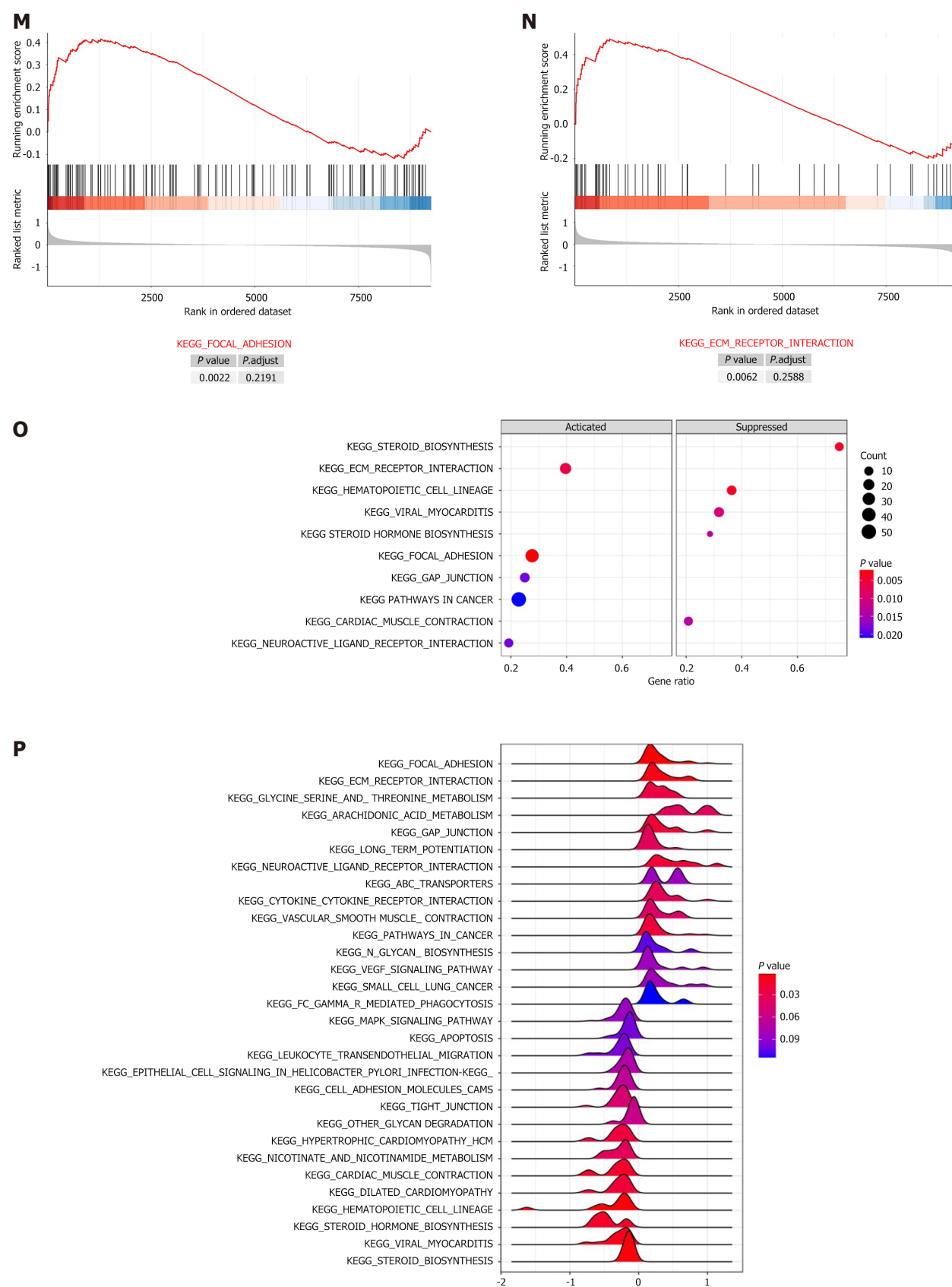
**DOI**: 10.4239/wjd.v14.i6.820 **Copyright** ©The Author(s) 2023.
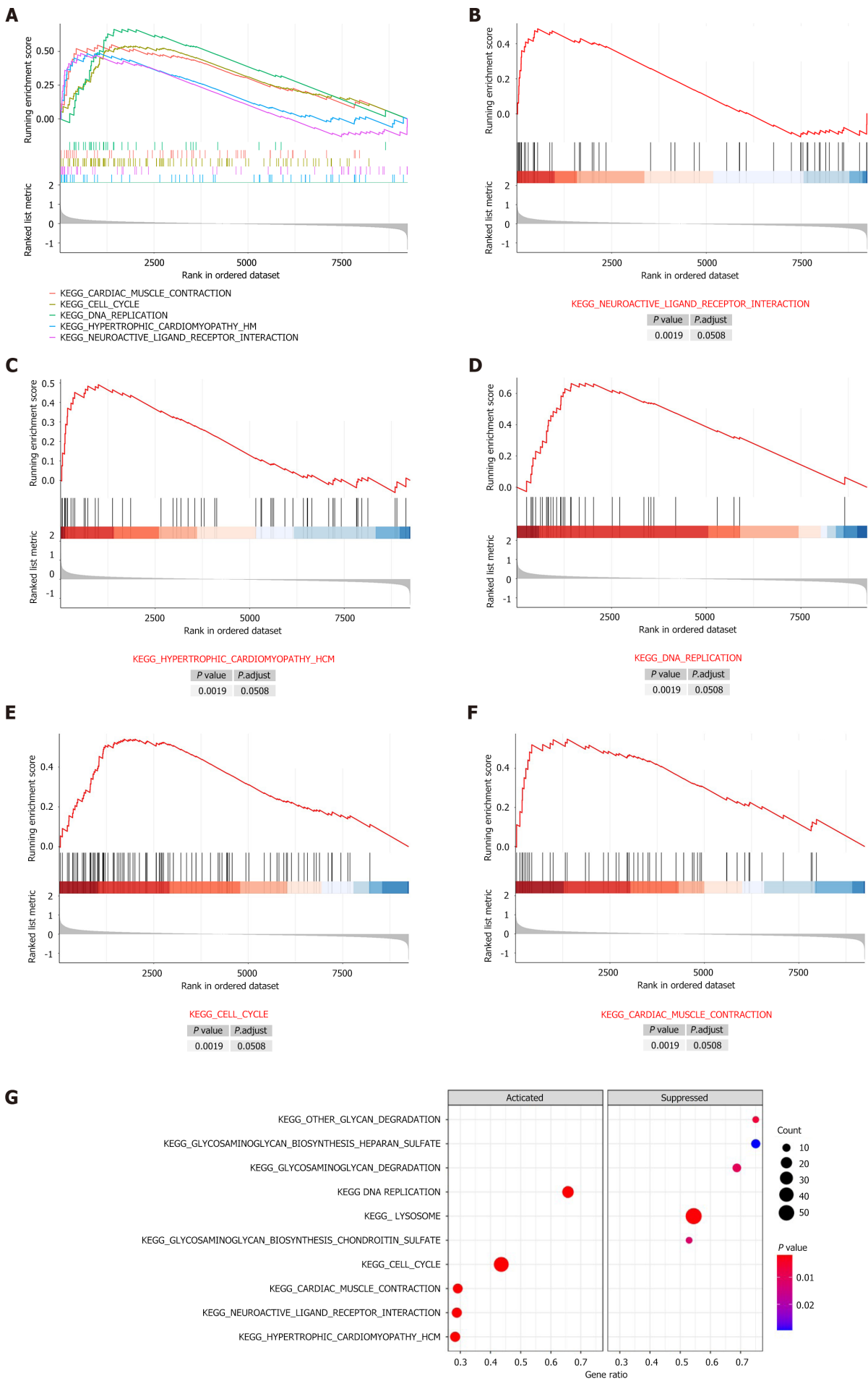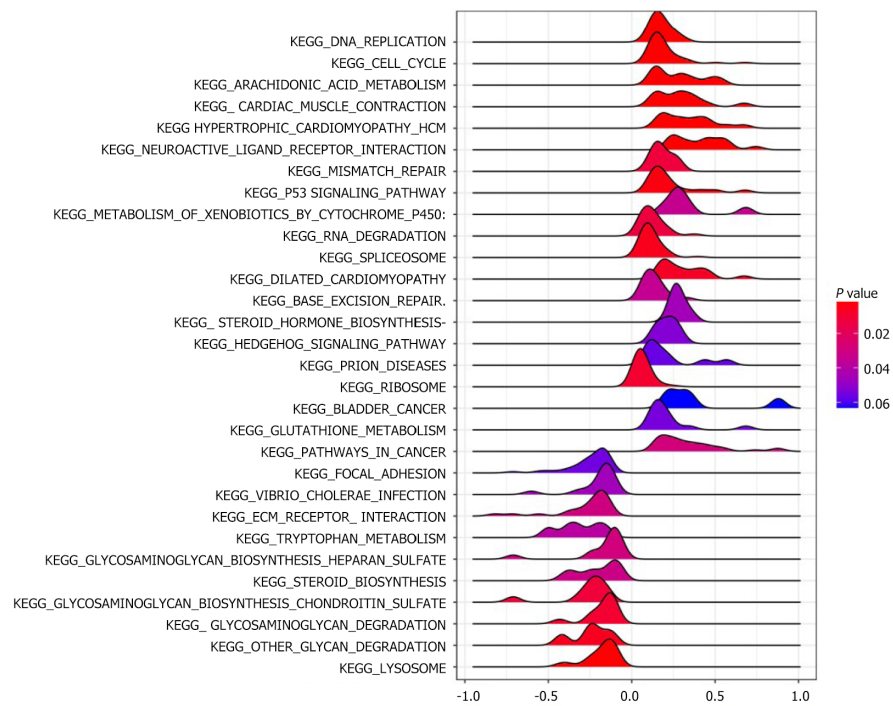
Figure 2 Gene set enrichment analysis. A: Top five gene set enrichment analysis in proliferating myoblasts; B: Neuroactive ligand–receptor interaction; C: Hypertrophic cardiomyopathy; D: DNA replication; E: Cell cycle; F: Cardiac muscle contraction; G: Bubble plot in proliferating myoblasts; H: Ridgeline plot in proliferating myoblasts; I: Top 5 gene set enrichment analysis in differentiated myotubes; J: Viral myocarditis; K: Steroid hormone biosynthesis; L: Hematopoietic cell lineage; M: Focal adhesion; N: Extracellular matrix–receptor interaction; O: Bubble plot in differentiated myotubes; P: Ridgeline plot in differentiated myotubes.
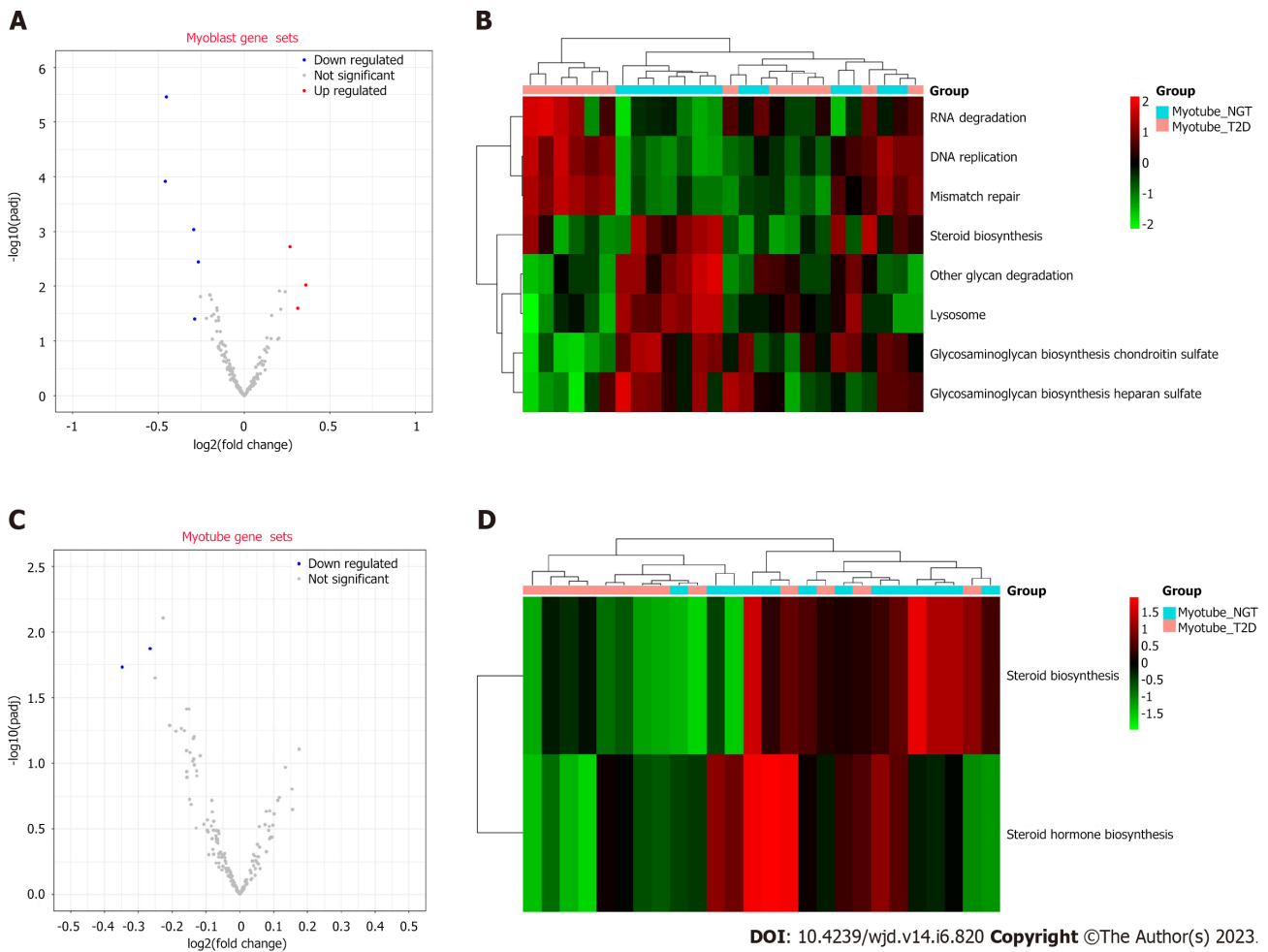
Figure 3 Gene set variation analysis. A: Volcano plot of proliferating myoblasts; B: Volcano plot of differentiated myotubes; C: Heat map of proliferating myoblasts; D: Heat map of differentiated myotubes.

stimulus, response to fatty acid, response to mechanical stimulus, respiratory tube development, cellular response to extracellular stimulus, response to alcohol, and cell–substrate adhesion (Figure 5A). The results indicated that numerous cellular components were involved in the collagen-containing ECM, ECM component, collagen trimer, ER lumen, ER–Golgi intermediate compartment, Golgi-associated vesicle membrane, the complex of collagen trimers, membrane raft, membrane microdomain, membrane region, phagocytic vesicle, neuronal cell body, Golgi-associated vesicle, focal adhesion, cell–substrate adherens junction, cell–substrate junction, postsynaptic endosome, transport vesicle, COPII-coated ER to Golgi transport vesicle, and ER to Golgi transport vesicle membrane (Figure 5B). The results indicated that numerous molecular functions were involved in prostaglandin receptor activity, ECM structural constituent, prostanoid receptor activity, icosanoid receptor activity, growth factor binding, ECM structural constituent conferring tensile strength, platelet-derived growth factor binding, transmembrane receptor protein kinase activity, heat shock protein binding, sulfur compound transmembrane transporter activity, transmembrane receptor protein tyrosine kinase activity, transmembrane-ephrin receptor activity, oxidoreductase activity, ephrin receptor activity, virus receptor activity, and hijacked molecular function (Figure 5C). Through KEGG function enrichment analysis, 26 pathways were significant, such as axon guidance, protein digestion and absorption, focal adhesion, protein processing in the ER, cortisol synthesis and secretion, Fc gamma R-mediated phagocytosis, renin secretion, glutathione metabolism, AMPK signaling pathway, ECM–receptor interaction, DNA replication, calcium signaling pathway, thyroid cancer, aldosterone synthesis, and secretion, lipid and atherosclerosis, P53 signaling pathway, other glycan degradation, biosynthesis of amino acids, ABC transporters, phospholipase D signaling pathway, and steroid biosynthesis (Figure 5D).

PPI analysis

The network consisted of 227 nodes and 416 edges (Figure 6A). We used the NetworkAnalyzer plugin to calculate the degree and combine the score (Figure 6B). We obtained 20 key genes (2 modules with 67 interactions) *via* the cytoHubba plugin (Table 1 and Figure 6C).
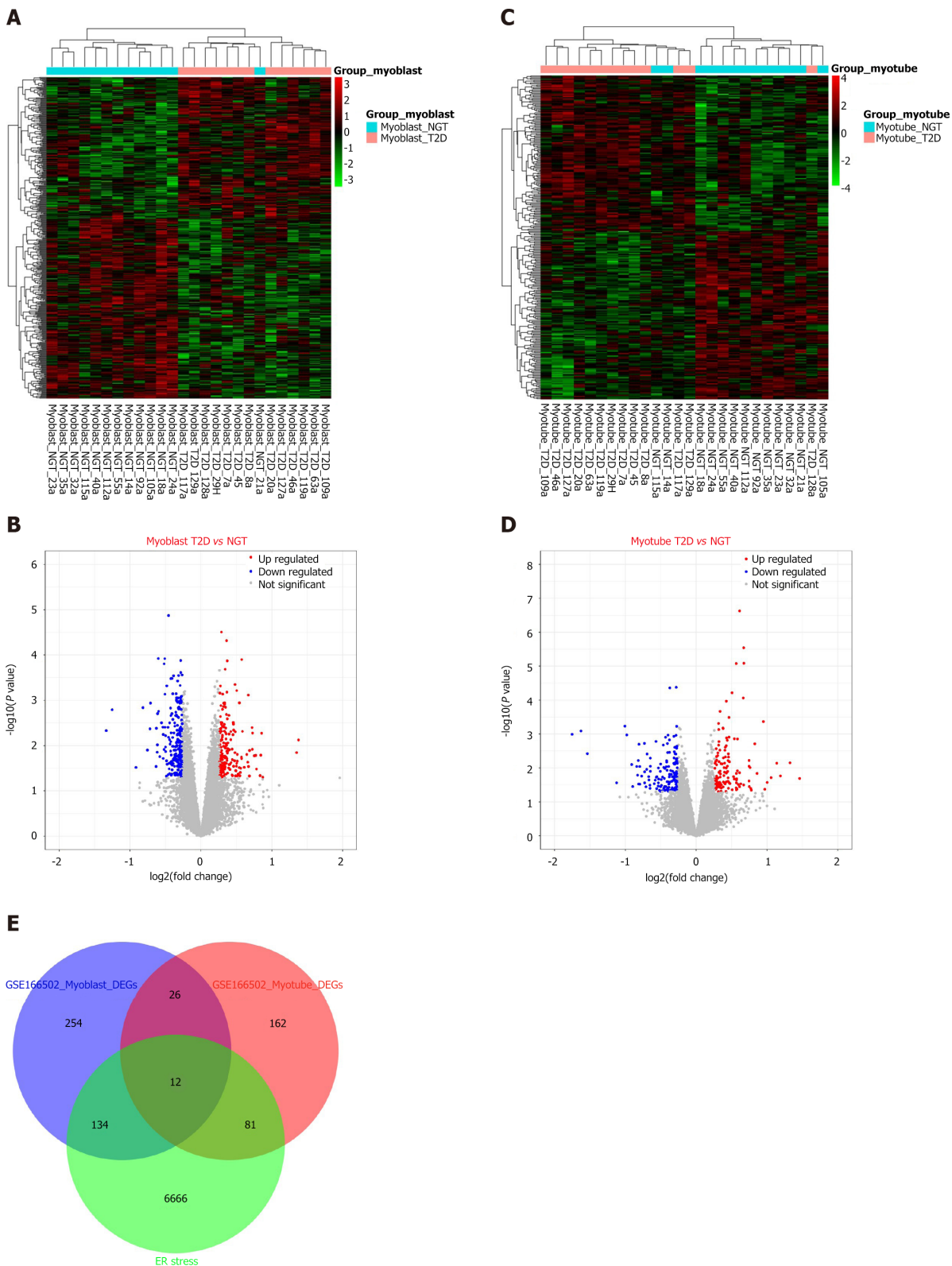
Figure 4 Endoplasmic reticulum stress-related differentially expressed genes. A: Heat map of proliferating myoblasts; B: Volcano plot of proliferating myoblasts; C: Heat map of differentiated myotubes; D: Volcano plot of differentiated myotubes; E: Endoplasmic reticulum stress-related differentially expressed genes. ER: Endoplasmic reticulum.

Network analysis

We obtained 27 TFs and 49 target genes from TRRUST to build the TF–mRNA network (Figure 7A). We obtained 51 miRNAs and 25 target genes from miRWalk to build the miRNA–mRNA network (Figure 7B). We also obtained 59 drugs and 22 target genes from DGIdb to build the drug–mRNA network (Figure 7C).

D
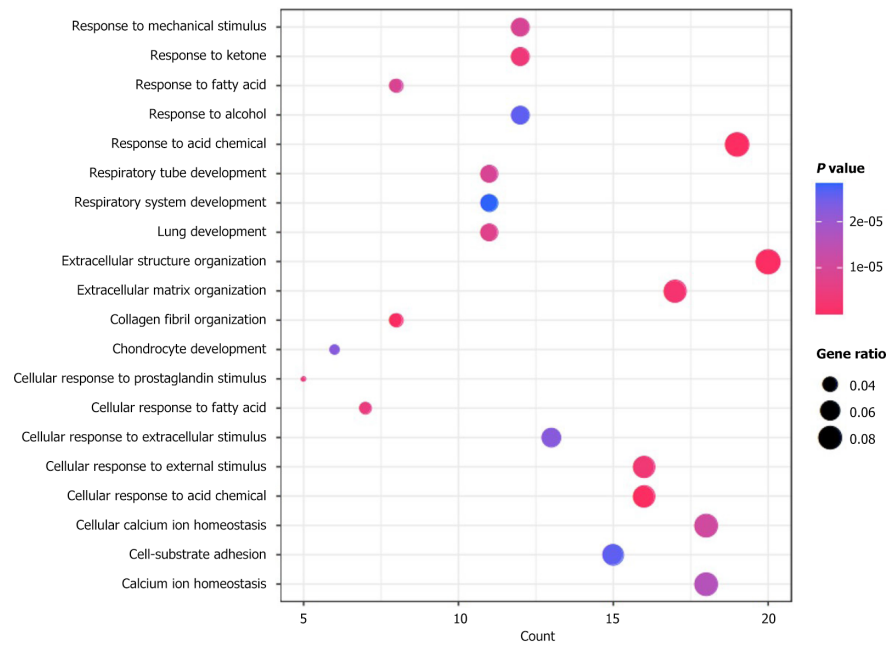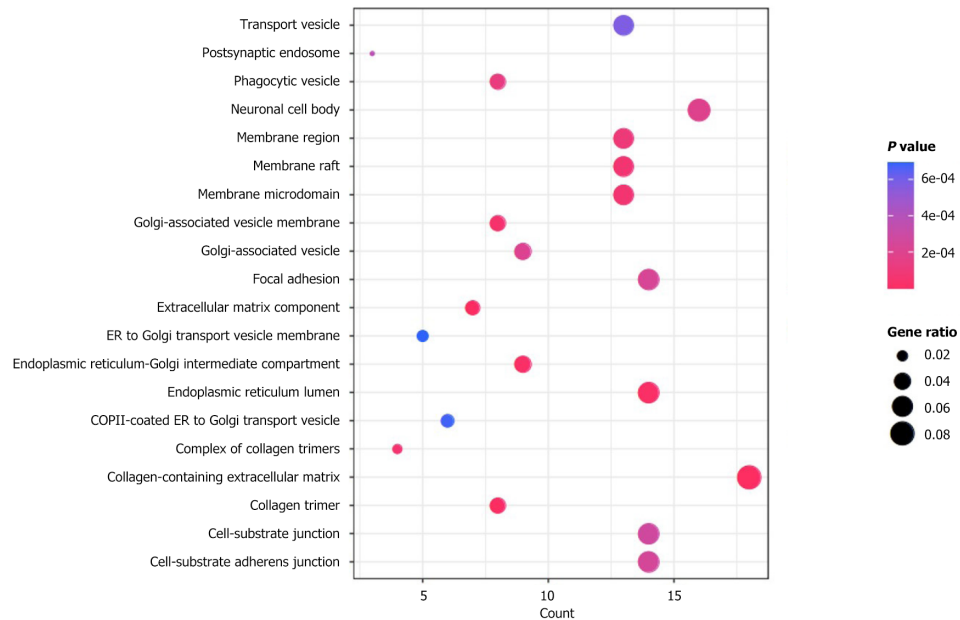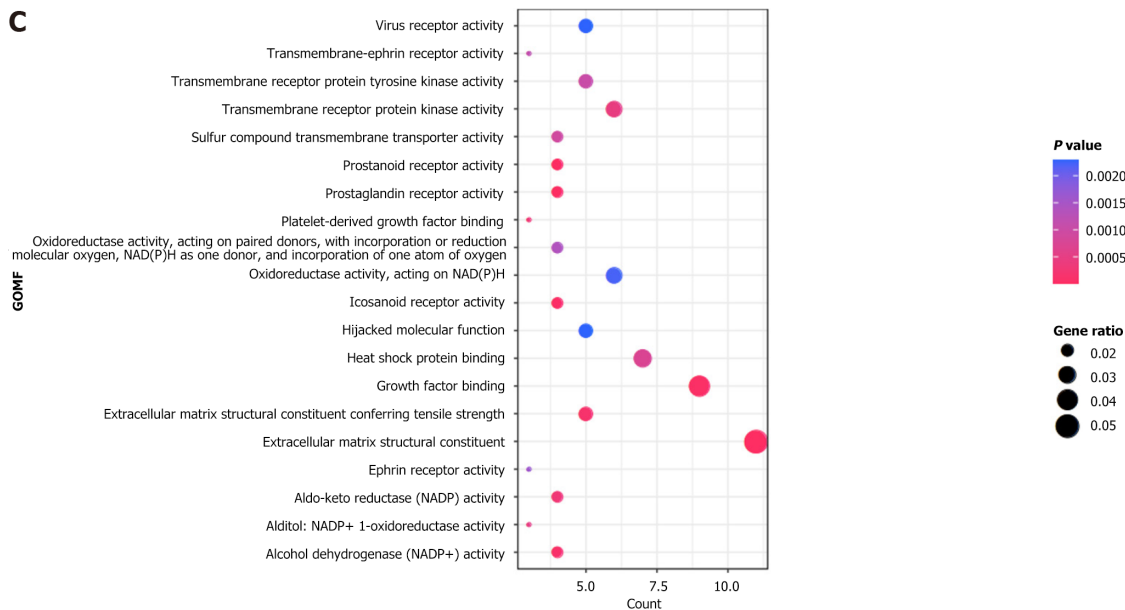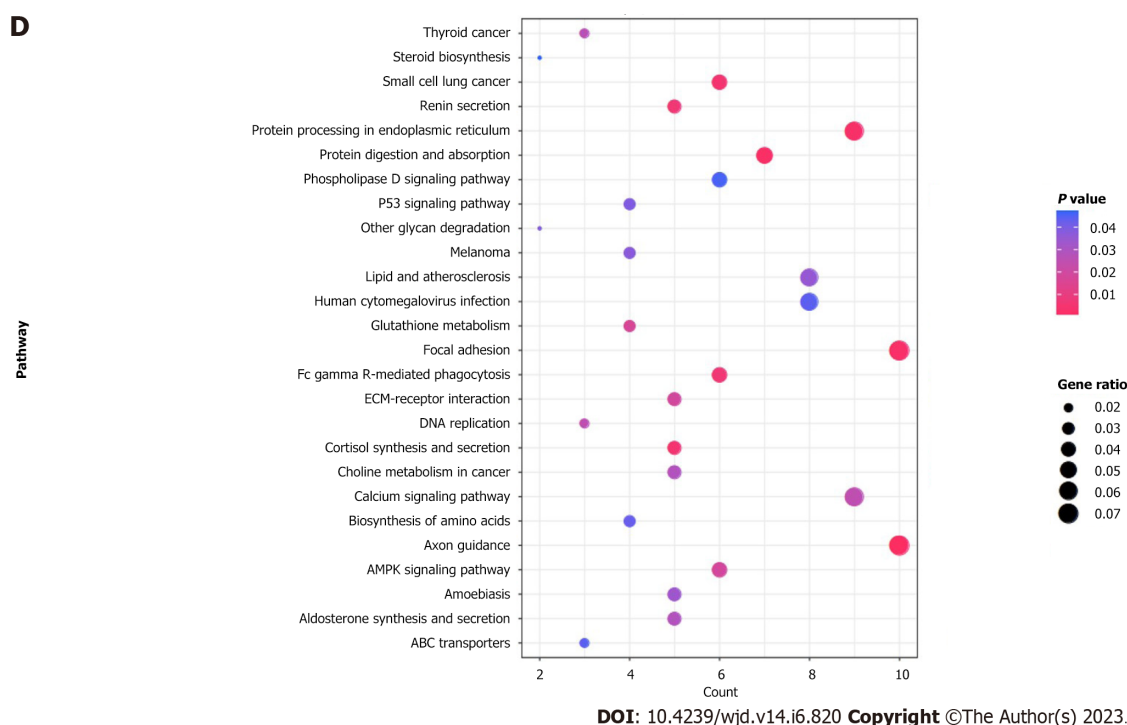
Figure 5 Functional enrichment analysis. A: Gene Ontogeny biological processes; B: Gene Ontogeny cellular components; C: Gene Ontogeny molecular function; D: Kyoto Encyclopedia of Genes and Genomes.

Correlation analysis of immune infiltration

We demonstrated that memory CD4^{+} T cells accounted for the largest proportion of 22 immune cell types (Figure 8A). Figure 8B showed the distribution of different immune cells in each sample. Moreover, we evaluated the correlation between immune infiltration and each sample (Figure 8C). In 20 key genes, the enrichment degree of each immune cell was different (Figure 8D).

DISCUSSION

T2DM is a complex metabolic disease driven by interactions among diverse environmental and genetic susceptibilities[39]. Although environmental and epigenetic factors clearly play a contributory role in the pathogenesis of T2DM, genetic factors appear to be the primary contributors to the recent rise in T2DM prevalence[40]. More studies have shown that ERS is involved in T2DM[41]. In the present study, we first explored the potential pathways in proliferating myoblasts and differentiated myotubes, and obtained 227 ERS-related DEGs in T2DM, which may contribute to the occurrence and development of T2DM. Later enrichment analysis, immune infiltration, TF–mRNA network, and miRNA–mRNA network revealed the mechanisms of T2DM, which provided a way for clinical treatment of T2DM. In particular, the drug–mRNA network provided new insights and perspectives into the therapeutic reagents.

In GSEA and GSVA, we confirmed that DNA replication, cell cycle, neuroactive ligand–receptor interaction, glycosaminoglycan biosynthesis heparan sulfate, glycosaminoglycan biosynthesis chondroitin sulfate, glycosaminoglycan degradation, other glycan degradation, lysosome, arachidonic acid metabolism, mismatch repair, metabolism of xenobiotics by cytochrome P450, steroid hormone biosynthesis, focal adhesion, and ECM–receptor interaction, neuroactive ligand–receptor interaction, gap junction, steroid biosynthesis, and cell adhesion molecules were enriched. Moreover, the P53 signaling pathway, VEGF signaling pathway, MAPK signaling pathway, and apoptosis may contribute to T2DM. Previous studies have indicated that these biological processes are related to T2DM[42-44]. SRT2104 enhanced renal SIRT1 expression and activity, deacetylated P53, and activated NRF2 antioxidant signaling, providing remarkable protection against T2DM[45]. The p-ERK/p-JNK/VEGF/ PKC signaling pathway may play an important role in pathological T2DM conditions[46]. TREM-2 negatively regulates p38 MAPK-mediated inflammatory response in T2DM[47]. These previous findings are consistent with our findings in this study.

We identified 227 ERS-related DEGs and later function enrichment analysis demonstrated that the enriched biological processes and pathways are highly consistent with the previous GSEA and GSVA results. The immune infiltration analysis revealed that memory CD4^{+} T cells accounted for the largest
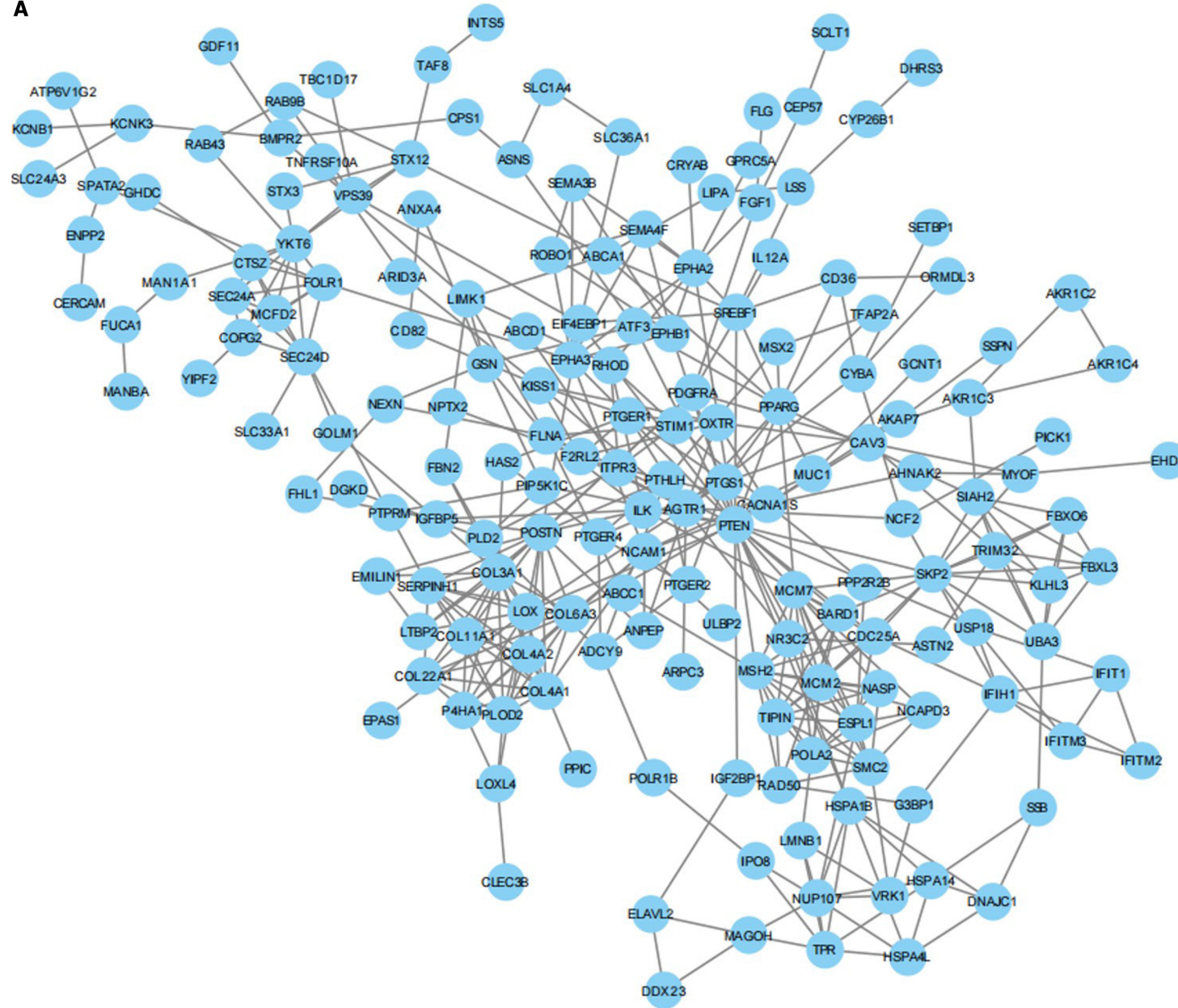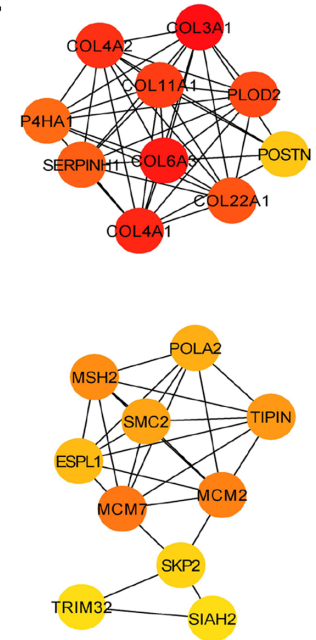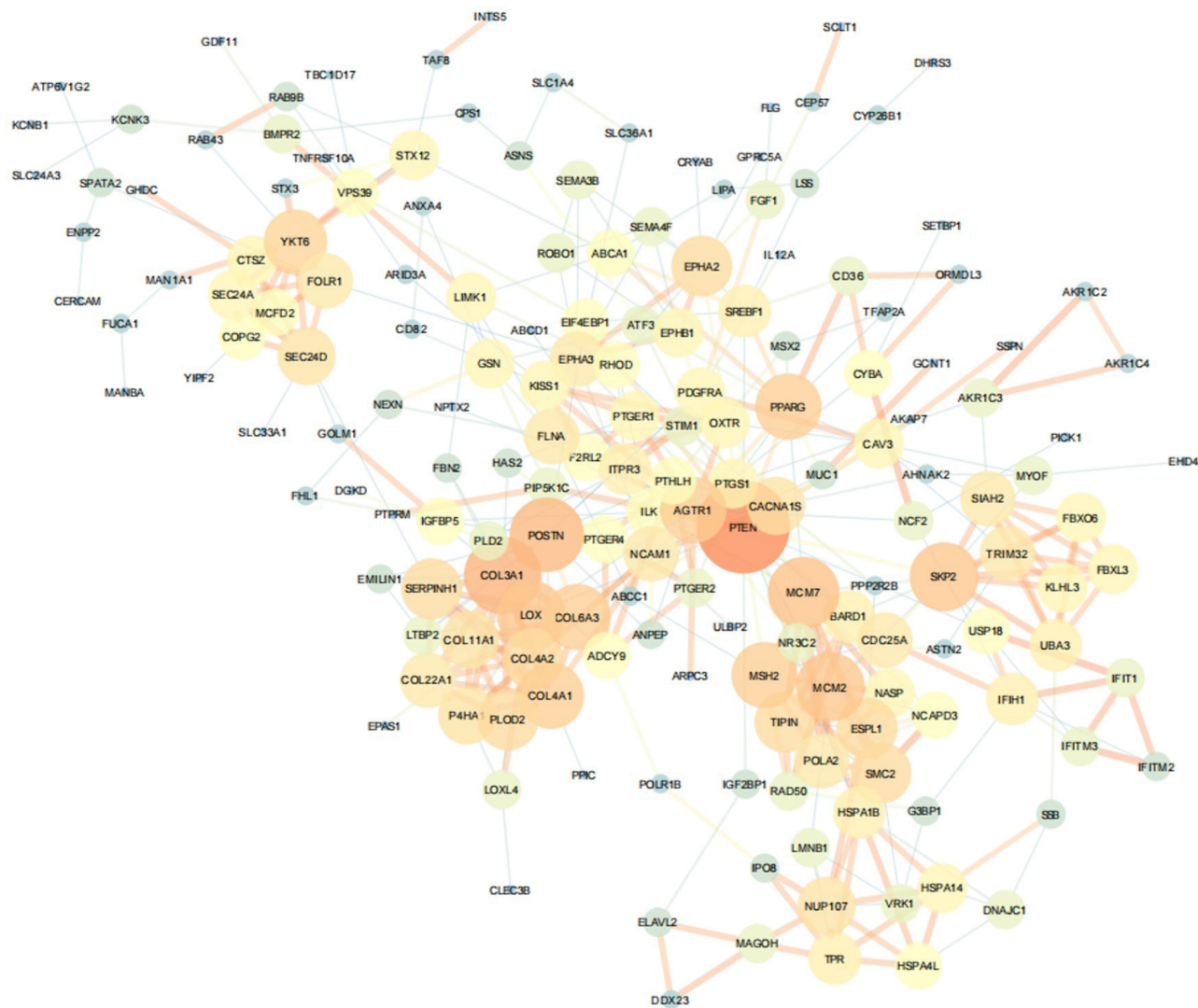
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Figure 6 Protein–protein interaction network. A: Protein–protein interaction (PPI) network; B: PPI network by NetworkAnalyzer; C: PPI network of 20 key genes.

Table 1 20 key genes

Gene	Description	MCC-score	Degree	Closeness	Betweenness
COL3A1	Collagen Type III Alpha 1 Chain	11179	16	64.5	1189.37723
COL6A3	Collagen Type VI Alpha 3 Chain	11168	12	62.03333	567.89507
COL4A1	Collagen Type IV Alpha 1 Chain	11167	12	59.91667	480.86034
COL4A2	Collagen Type IV Alpha 2 Chain	10806	10	56.23333	84.68666
COL11A1	Collagen Type XI Alpha 1 Chain	10800	9	54.5	18.34225
PLOD2	Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 2	10326	10	56.88333	112.4573
COL22A1	Collagen Type XXII Alpha 1 Chain	10080	8	51.80952	4.88333
SERPINH1	Serpin Family H Member 1	6001	10	60.3	1308.02191
P4HA1	Prolyl 4-Hydroxylase Subunit Alpha 1	5166	9	55.07857	68.74063
MCM7	Minichromosome Maintenance Complex Component 7	1804	14	68.24286	1418.45764
MCM2	Minichromosome Maintenance Complex Component 2	1801	13	63.94524	784.74991
MSH2	MutS Homolog 2	1645	12	62.22619	646.66407
TIPIN	TIMELESS Interacting Protein	1596	10	54.1631	37.22401
SMC2	Structural Maintenance of Chromosomes 2	1567	10	53.77976	230.10198
POLA2	DNA Polymerase Alpha 2, Accessory Subunit	1560	8	52.6131	4.22778
ESPL1	Extra Spindle Pole Bodies Like 1, Separase	962	10	56.52976	455.86734
POSTN	Periostin	860	15	66.51667	1995.77049
SKP2	S-Phase Kinase Associated Protein 2	771	13	64.5619	1861.813
TRIM32	Tripartite Motif Containing 32	722	8	54.52976	282.93712
SIAH2	Siah E3 Ubiquitin Protein Ligase 2	722	8	57.06905	982.96735

MCC: McCormick.

proportion of 22 immune cell types. T2DM patients are present with self-reactive T cells with a memory phenotype[48]. The memory CD4+ T cells develop directly from effector cells and thereby preserve features of their effector precursors are reserved[49]. Depending on the immune context, memory CD4+ T cells can contribute to immune protection, pathology, or tissue remodeling[50]. The memory CD4+ T cells could act as immunological markers for predicting change in β-cell function in T2DM[51]. TFs recognize specific DNA sequences to control chromatin and transcription, forming a complex system that guides the expression of the genome[52]. Here we obtained 27 TFs, which may contribute to T2DM. MiRNA is a class of endogenous noncoding RNA encoding 19–25 nucleotides, which is involved in the post-transcriptional regulation of genes[53]. Most of them have high sequence conservation, expression timing, and tissue specificity[54]. Recent studies have shown that miRNA is involved in a variety of regulatory pathways, we here identified 51 miRNAs to further explain the mechanisms of T2DM. Importantly, we also established a drug–mRNA network map to provide new ideas and directions for the treatment of T2DM. Immune infiltration plays an important role in the occurrence and development of T2DM[55,56]. The memory CD4+ T cells play central roles in immunity in health and disease[57]. We also explored the relationship between immune infiltration and T2DM, and we found that memory CD4+ T cells were the most numerous types of immune cells in T2DM. Previous studies indicated that CD4+ T cells contribute to the destruction of insulin-producing β-cells in type 1 diabetes mellitus[58,59], which confirmed our results.

This study has some limitations. First, all the results of the analysis were derived from previous data. Despite the efforts we have made in the present, our results still need verification experimentally and clinically. Moreover, the TF–mRNA, miRNA–mRNA, and drug–mRNA networks we built in this study provided some new ideas and insights for the mechanisms and treatment of T2DM. However, this is only the beginning, and more work is still needed in the follow-up.

A

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B

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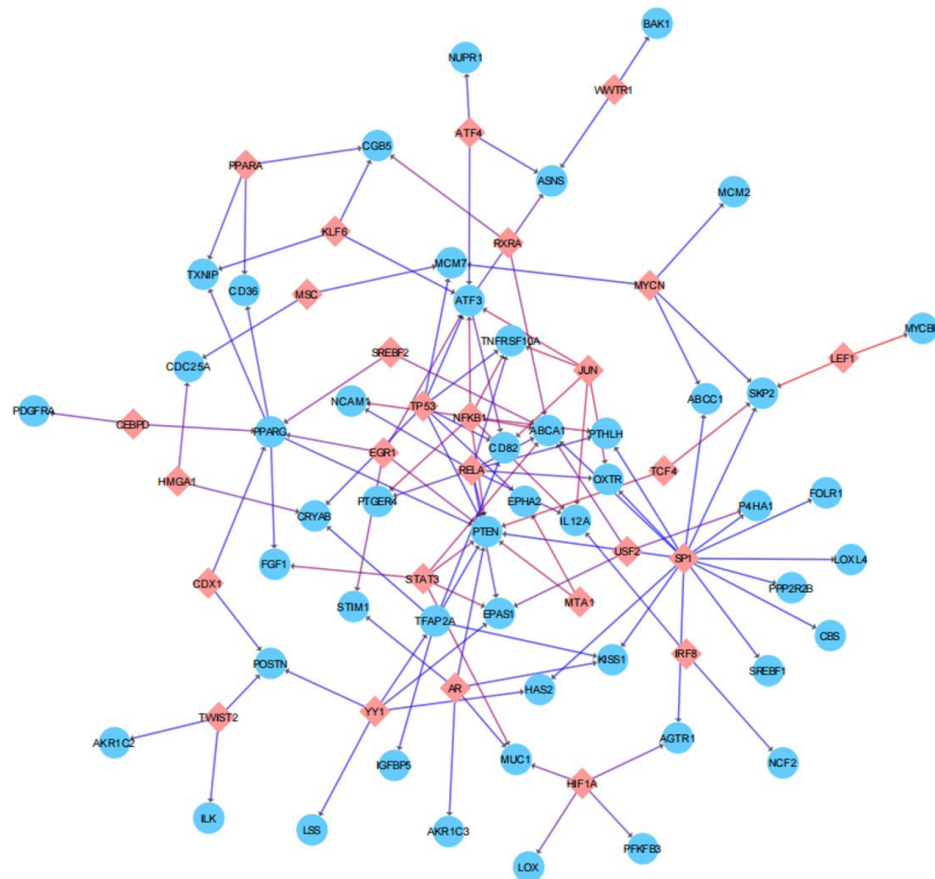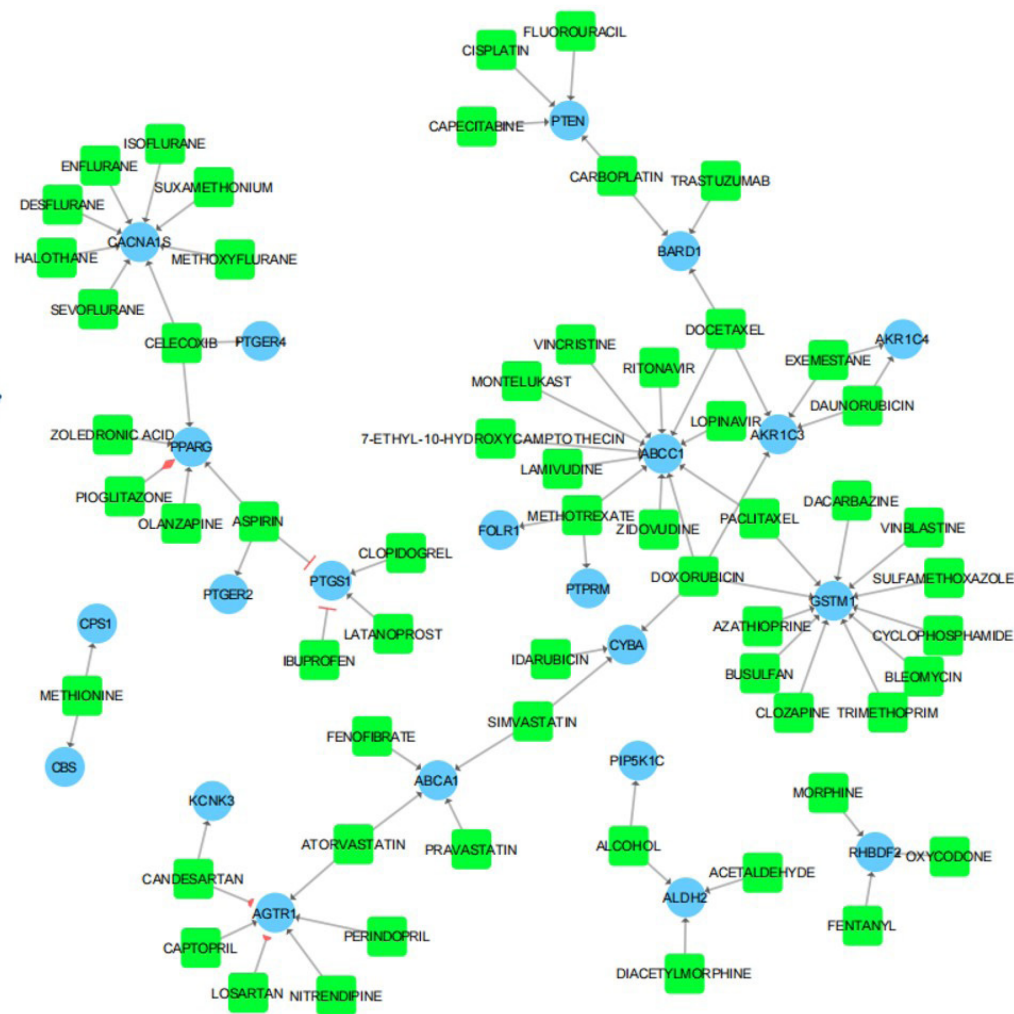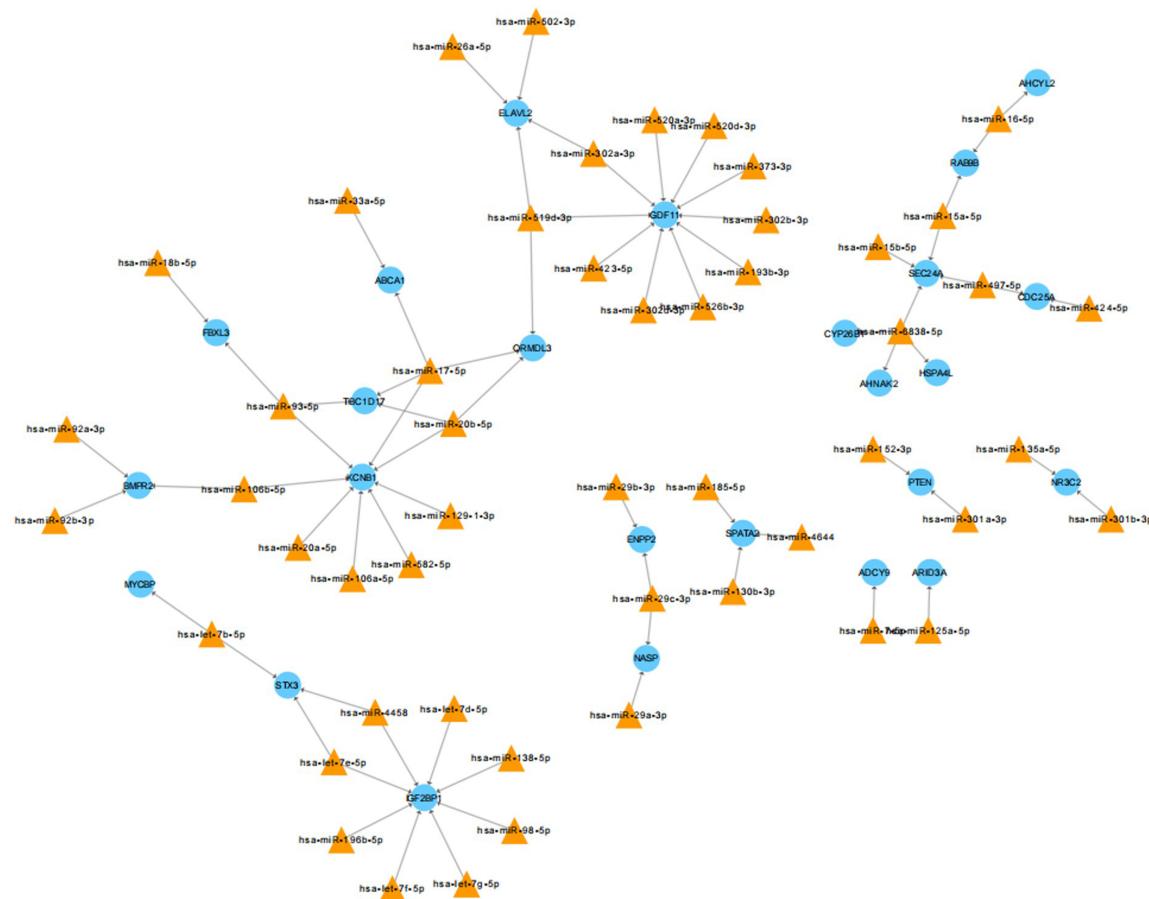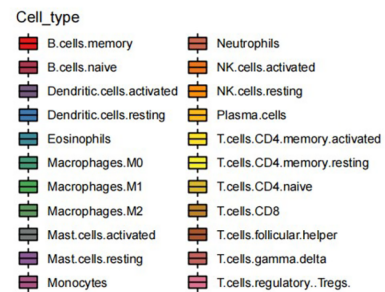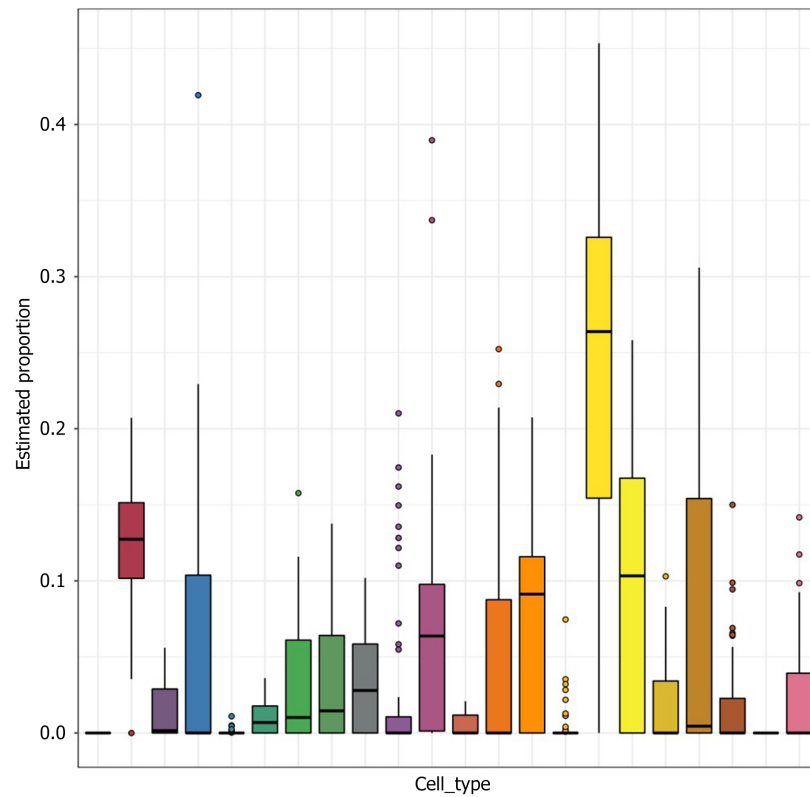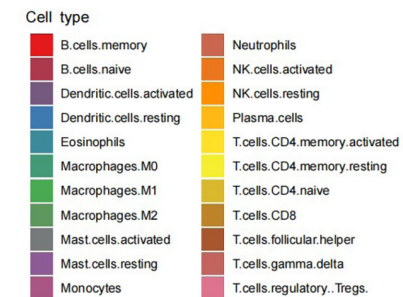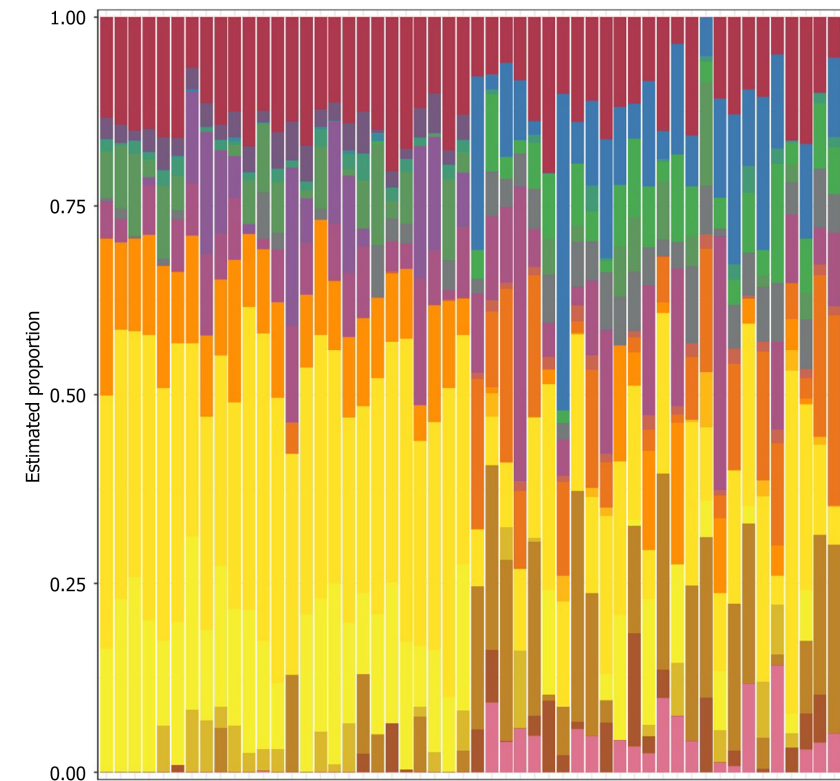
Figure 7 Networks. A: Transcription factor–mRNA network; B: miRNA–mRNA network; C: Drug–mRNA network.

CONCLUSION

This study revealed ERS-related mechanisms in T2DM, which might contribute to new ideas and insights for the mechanisms and treatment of T2DM.
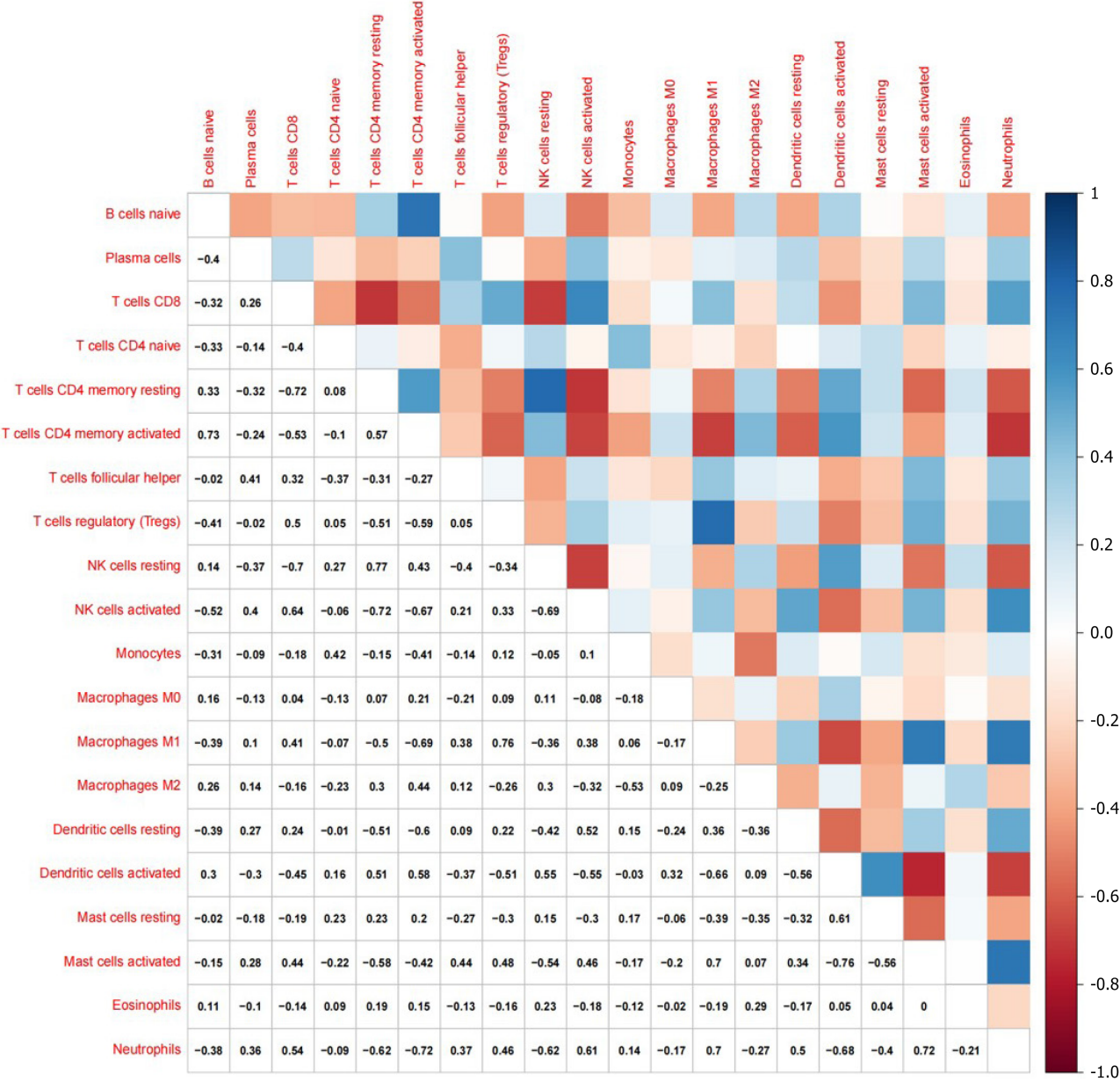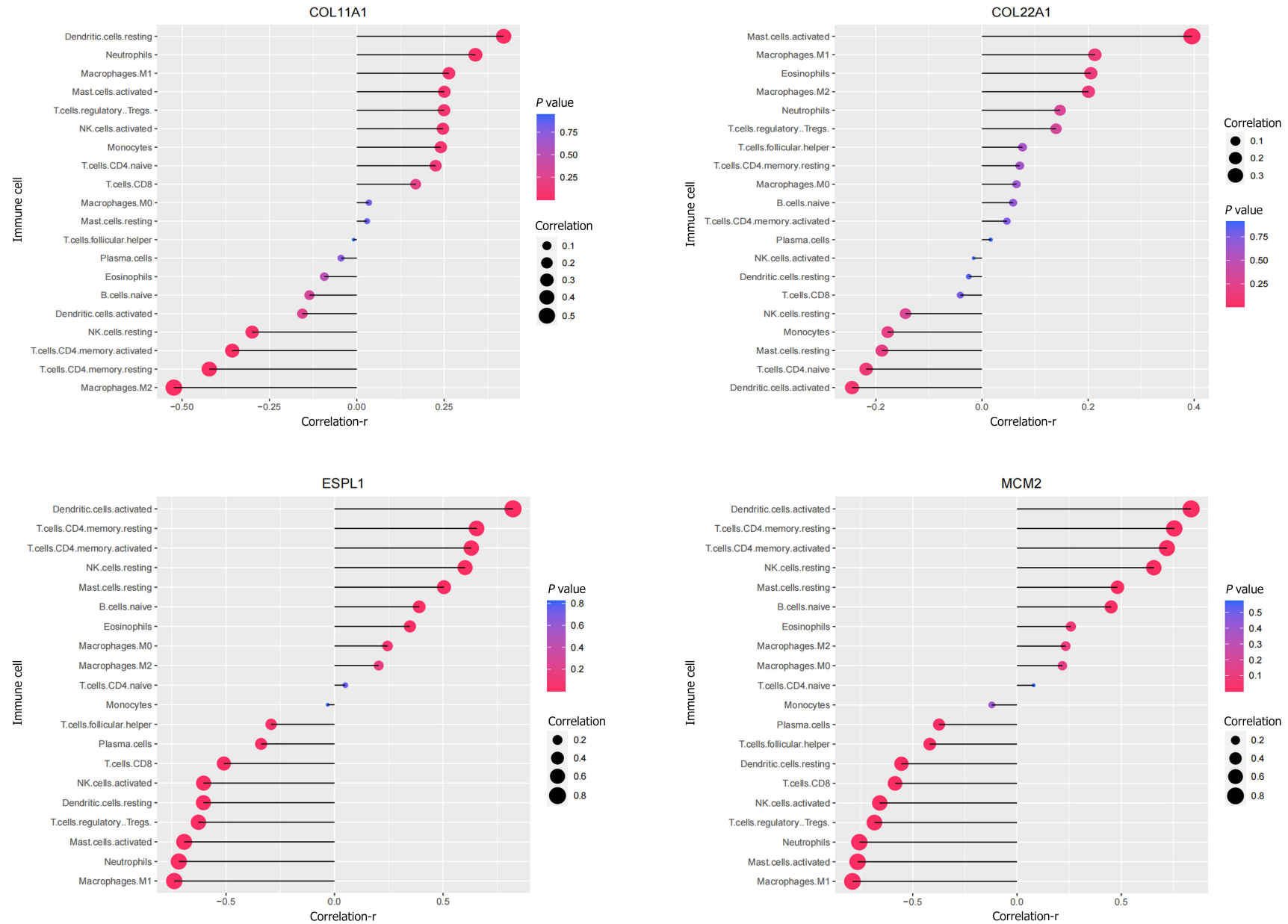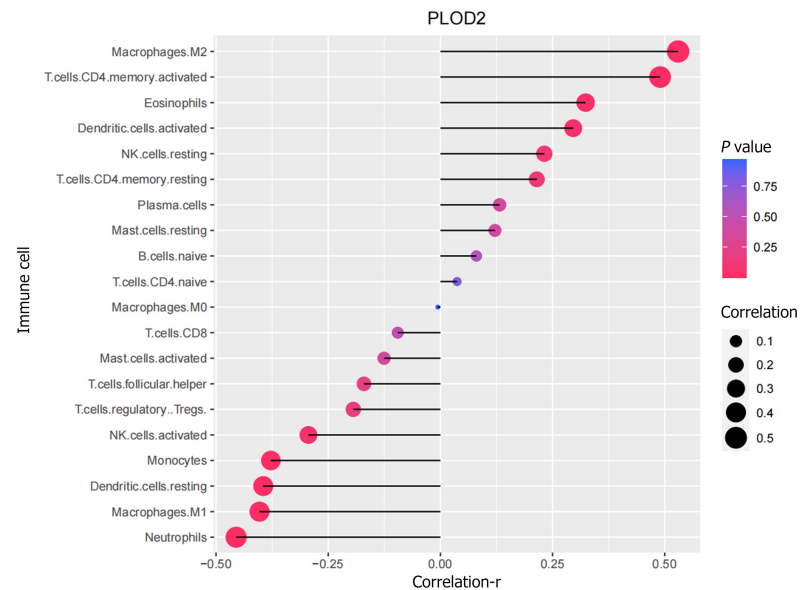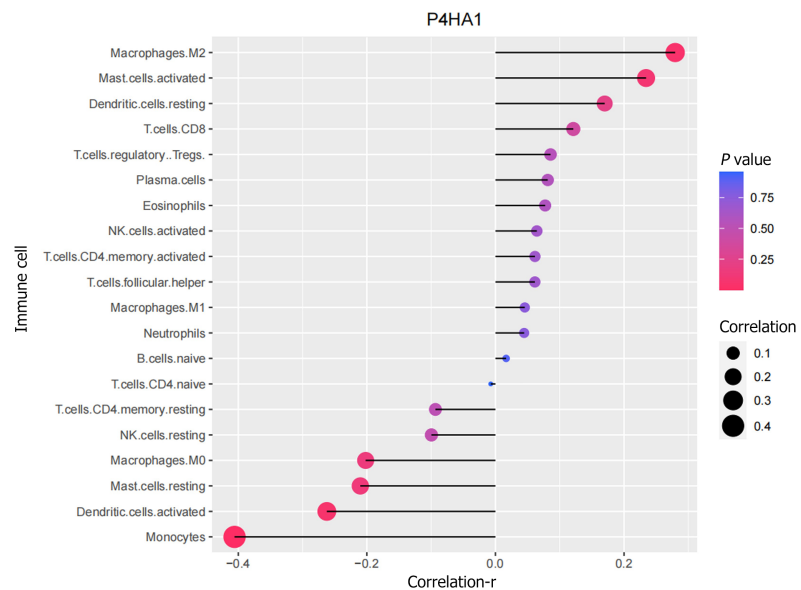
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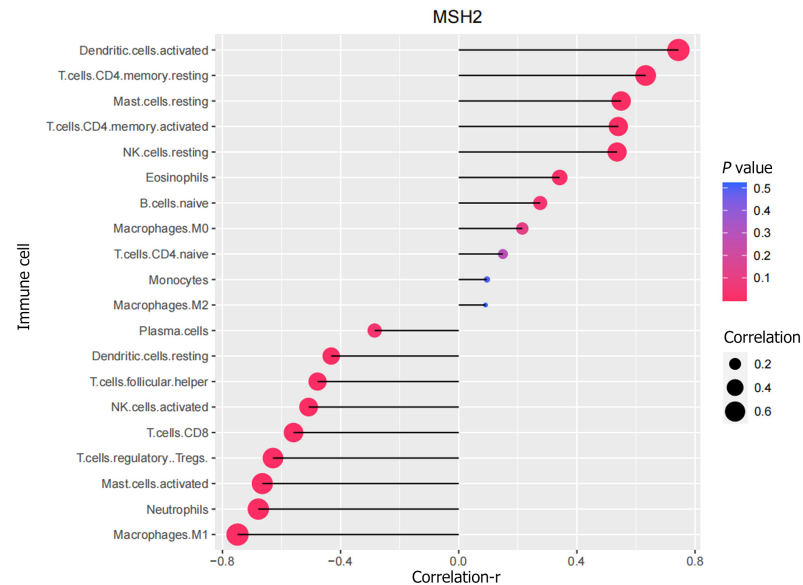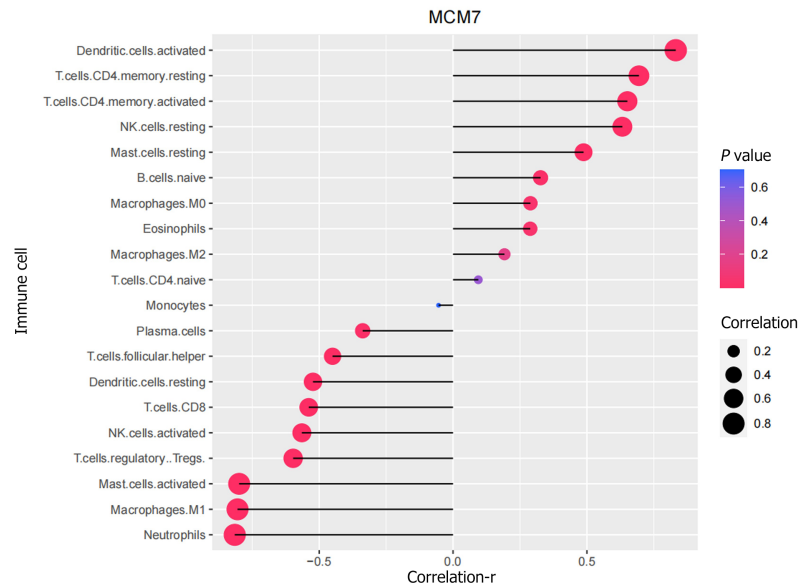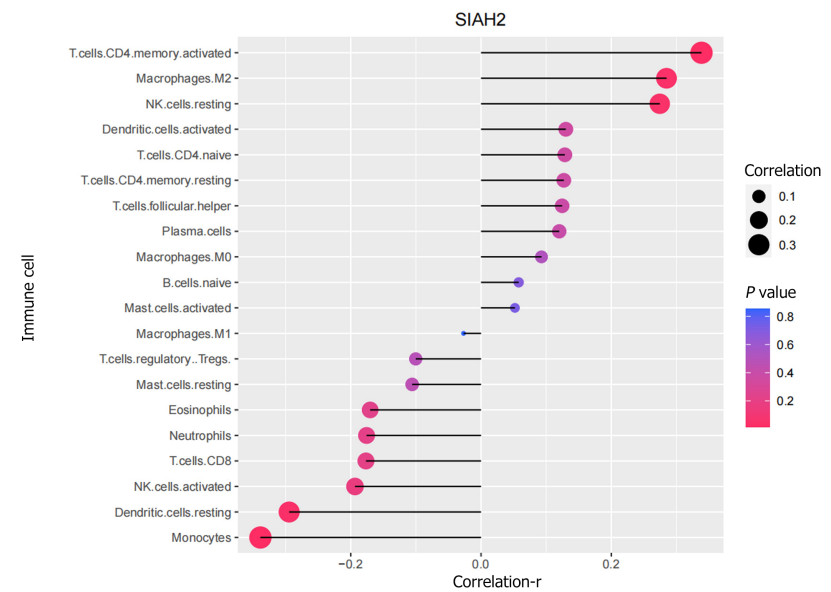
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Figure 8 Immune infiltration. A: Histogram of immune infiltration distribution; B: Histogram of immune infiltration sample distribution; C: Heat map of immune infiltration correlation; D: Correlation diagram of 20 key genes.

ARTICLE HIGHLIGHTS

Research background

The endoplasmic reticulum (ER) is closely related to a wide range of cellular functions and is a key component to maintain and restore metabolic health.

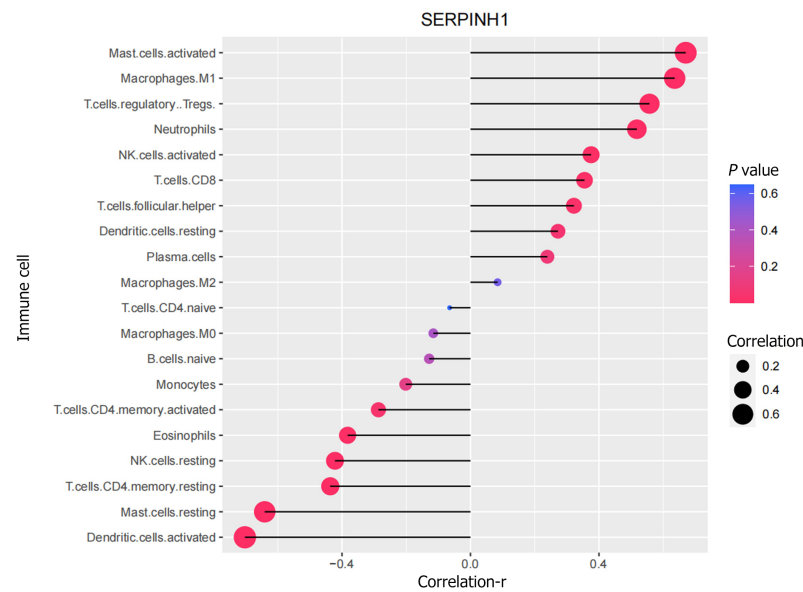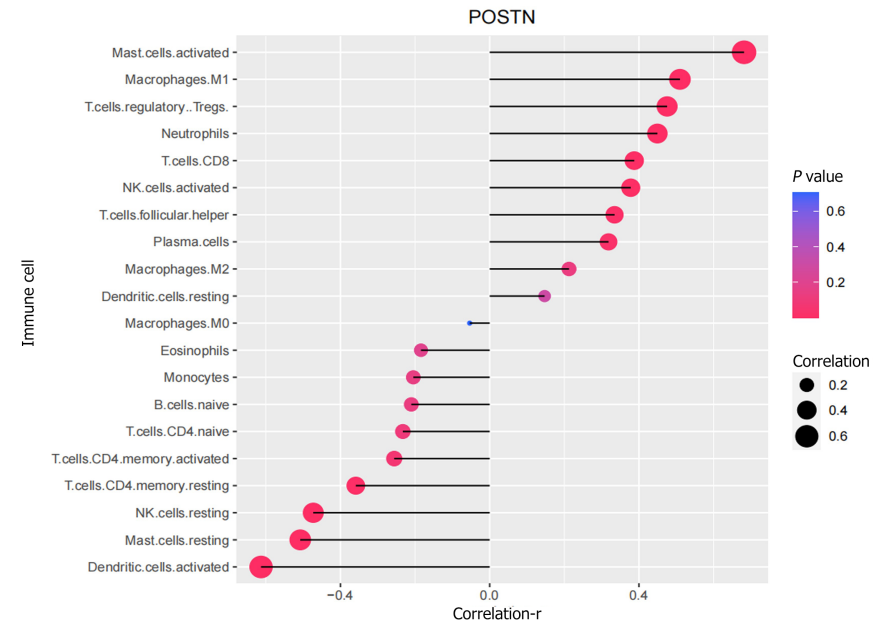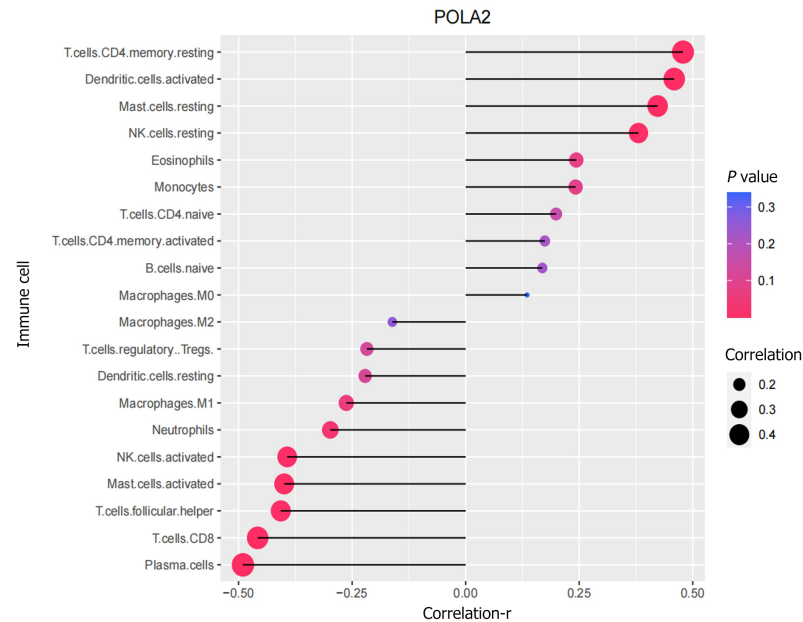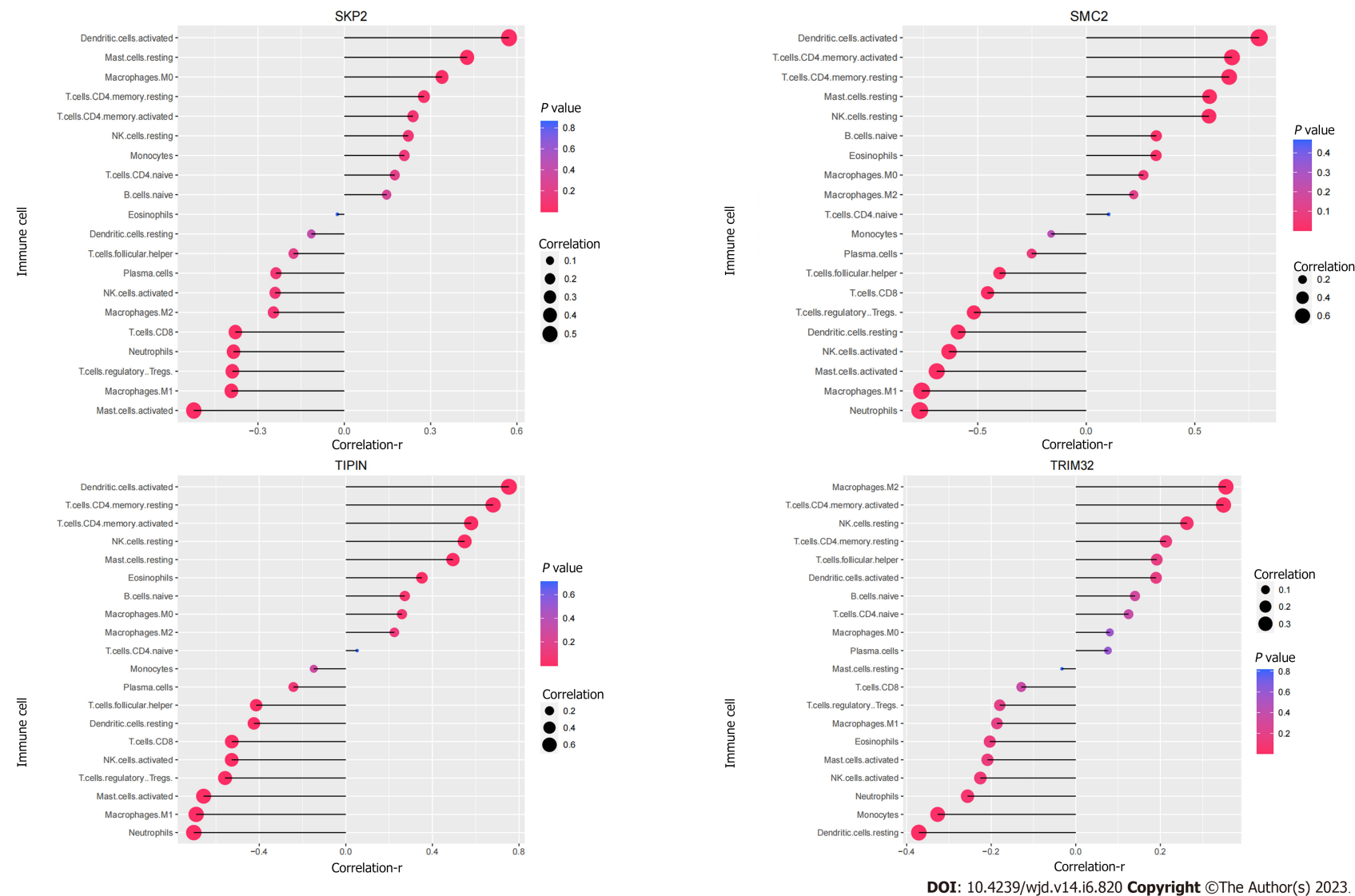### Research motivation

Type 2 diabetes mellitus (T2DM) is a serious threat to human health, but knowledge of the ER stress (ERS)-related mechanisms in T2DM is lacking.

Research objectives

Here, we conducted a bioinformatics analysis to identify potential ERS-related mechanisms and crucial biomarkers in T2DM.

Research methods

We conducted gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) in myoblast and myotube form GSE166502, and obtained the differentially expressed genes (DEGs). After intersecting with ERS-related genes, we obtained ERS-related DEGs. Finally, functional analyses, immune infiltration, and several networks were established.

Research results

Through GSEA and GSVA, we identified several metabolic and immune-related pathways. We obtained 227 ERS-related DEGs and constructed several important networks that help to understand the mechanisms and treatment of T2DM. Finally, memory CD4+ T cells accounted for the largest proportion of immune cells.

Research conclusions

This study revealed ERS-related mechanisms in T2DM.

Research perspectives

Our study might contribute to new ideas and insights for the mechanisms and treatment of T2DM.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Lomatogonium rotatum extract alleviates diabetes mellitus induced by a high-fat, high-sugar diet and streptozotocin in rats

Li-Li Dai, Sung-Bo Cho, Hui-Fang Li, Li-Sha A, Xiao-Ping Ji, Sirigunqiqige Pan, Ming-Lan Bao, Laxinamujila Bai, Gen-Na Ba, Ming-Hai Fu

Li-Li Dai, Sung-Bo Cho, Hui-Fang Li, Xiao-Ping Ji, Sirigunqiqige Pan, Ming-Lan Bao, Laxinamujila Bai, Gen-Na Ba, Ming-Hai Fu, NMPA Key Laboratory of Quality Control of Traditional Chinese Medicine (Mongolian Medicine), Inner Mongolia Minzu University, Tongliao 028000, Inner Mongolia Autonomous Region, China

Li-Sha A, Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Provincial Key Laboratory for Research and Development of Tropical Herbs, Hainan Medical University, Haikou 571199, Hainan Province, China

Ming-Hai Fu, Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Provincial Key Laboratory for Research and Development of Tropical Herbs, School of Pharmacy, Hainan Medical University, Haikou 571199, Hainan Province, China

Corresponding author: Ming-Hai Fu, PhD, Associate Professor, NMPA Key Laboratory of Quality Control of Traditional Chinese Medicine (Mongolian Medicine), Inner Mongolia Minzu University, No. 996 Xilamulun Street, Horqin District, Tongliao 028000, Inner Mongolia Autonomous Region, China. mfu@imun.edu.cn

Abstract

BACKGROUND

Lomatogonium rotatum (LR) is traditionally used in Mongolian folk medicine as a hypoglycemic agent, but its evidence-based pharmacological effects and mechanisms of action have not been fully elucidated.

AIM

To emphasize the hypoglycemic action mechanism of LR in a type 2 diabetic rat model and examine potential biomarkers to obtain mechanistic understanding regarding serum metabolite modifications.

METHODS

A high-fat, high-sugar diet and streptozotocin injection-induced type 2 diabetic rat model was established. The chemical composition of the LR was identified by high performance liquid chromatography. LR extract administrated as oral gavage at 0.5 g/kg, 2.5 g/kg, and 5 g/kg for 4 wk. Anti-diabetic effects of LR extract were evaluated based on histopathological examination as well as the measurement of blood glucose, insulin, glucagon-like peptide 1 (GLP-1), and lipid levels. Serum metabolites were analyzed using an untargeted metabolomics

approach.

RESULTS

According to a chemical analysis, swertiamarin, sweroside, hesperetin, coumarin, 1.7-dihydroxy-3,8-dimethoxyl xanthone, and 1-hydroxy-2,3,5 trimethoxanone are the principal active ingredients in LR. An anti-diabetic experiment revealed that the LR treatment significantly increased plasma insulin and GLP-1 levels while effectively lowering blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, and oral glucose tolerance test compared to the model group. Furthermore, untargeted metabolomic analysis of serum samples detected 236 metabolites, among which 86 were differentially expressed between the model and the LR group. It was also found that LR considerably altered the levels of metabolites such as vitamin B6, mevalonate-5P, D-proline, L-lysine, and taurine, which are involved in the regulation of the vitamin B6 metabolic pathway, selenium amino acid metabolic pathway, pyrimidine metabolic pathway, and arginine and proline metabolic pathways.

CONCLUSION

These findings indicated that LR may have a hypoglycemic impact and that its role may be related to changes in the serum metabolites and to facilitate the release of insulin and GLP-1, which lower blood glucose and lipid profiles.

Key Words: Mongolian medicine; *Lomatogonium rotatum*; Type 2 diabetes; Metabolomics; Swertiamarin; Streptozotocin

Core Tip: *Lomatogonium rotatum* (LR) is traditionally used in Mongolian folk medicine as a hypoglycemic agent. Its evidence-based pharmacological effects and mechanisms of action have not been elucidated. An anti-diabetic experiment in rats revealed that LR treatment increased insulin and glucagon-like peptide 1 levels and decreased blood sugar, total cholesterol, triglycerides, low-density lipoprotein cholesterol, and oral glucose tolerance test. These findings indicated that LR may have a hypoglycemic impact and that its role may be related to changes in the serum metabolites as well as to facilitating the release of insulin and glucagon-like peptide 1, which lower blood glucose and lipid profiles.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by chronically elevated blood glucose (BG) (hyperglycemia) and elevated blood insulin (hyperinsulinemia)[1]. T2DM is treated primarily with six classes of anti-diabetic medications, including metformin, glimepiride, repaglinide, pioglitazone, sitagliptin, and acarbose[2]. Traditional medicine has a long history of use as a complementary alternative therapy and has shown promising results in treating T2DM. The demand for complementary and alternative medicine has increased owing to its potential to target a multitude of metabolic pathways for treating T2DM.

Lomatogonium rotatum (LR) is a dried whole herb derived from the Gentianaceae plant *Lomatogonium rotatum* (L.) Fries ex Nym and is an important medicinal herb utilized in the formulation and practice of Mongolian medicine in China[3]. According to a previous study, LR could decrease the body weight of obese rats induced by a high-fat high-sugar (HFHS) diet[4]. The LR compounds can activate the bitter taste receptors, which have advantageous effects on diabetes[5,6]. The main compounds of LR include flavonoids and xanthones, small amounts of iridoids, alkaloids, steroids, and organic acids[6-9]. Nonetheless, the protective effects of LR against diabetes have not been thoroughly examined.

It is widely known that T2DM comprises several abnormalities in the systemic metabolism of amino acids (AAs), lipids, and glucose[10,11]. The metabolites associated with food metabolism provide a direct functional reading of an organism's physiological condition. Metabolomics analysis is the untargeted identification and quantification of all low molecular weight metabolic end products

(metabolites)[12]. Metabolomics technology is used to investigate the impact of drugs on endogenous metabolite variations and to identify specific biomarkers and their key factors[13]. Moreover, it provides a perspective image of downstream gene expression and vital information regarding drug metabolism [14]. Metabolic profiles of cells, tissues, organs, and biological fluids can be used to infer an individual's health status and help monitor changes in specific diseases[15]. In recent years, metabolomics has been used to systematically study the metabolites of patients with T2DM and find biomarkers and possible metabolic pathways. The dynamic changes of endogenous metabolites are closely related to the occurrence and development of diabetes. Understanding the hypoglycemic effect of LR on T2DM, identifying its biomarkers, and clarifying its mechanism by metabolomic studies will have considerable clinical significance.

MATERIALS AND METHODS

Materials
The HFHS diet was provided by Liaoning Changsheng Biotechnology Co., Ltd. (Shenyang, China; Batch No. 20200925). Analytical citric acid and sodium citrate were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). BG, insulin, glucagon-like peptide 1 (GLP-1), total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) kits were provided by Shenzhen Icubio Biotechnology Co., Ltd. (Shenzhen, China). Hematoxylin and eosin (H&E) stain was obtained from Nanjing Jiancheng Technology Co., Ltd. (Nanjing, China).

Preparation of LR extract
LR was collected from Xilinhaote grassland, Inner Mongolia, China. Five kilograms of LR was washed, dried, and powdered. Then, LR powder was extracted three times with 95% ethanol for 3 h each time. The extract was combined, concentrated, and freeze-dried at 60 °C under a vacuum. Carboxymethylcellulose sodium salt solvent was employed to suspend the LR extract. Animals received LR at 0.5 g/kg, 2.5 g/kg, and 5 g/kg concentrations by oral gavage according to the previous report[16].

Animals and experimental design
SPF grade male Sprague-Dawley rats (batch no. C-NMG2021012507), aged 6-8 wk (initial body weight of 180-220 g), were obtained from Changsheng Biotechnology Co., Ltd (Shenyang, China). Rats were kept individually in the SPF standard animal room with 30%-40% humidity, 22-25 °C temperature, and a 12-h light/dark cycle. After adaptive feeding for 1 wk, rats were randomly assigned to six groups: control group; model group; LR-0.5 group; LR-2.5 group; LR-5 group; and metformin group (a clinical anti-diabetic drug). The HFHS diet (30% lard oil, 20% sucrose, and 50% standard diet) was fed to the diabetic model for 4 wk along with an injection of streptozotocin (STZ) (30 mg/kg)[17], whereas the control animals received a commercial standard diet. After modeling, rats in the control and dietetic groups received 0.5% carboxymethylcellulose sodium salt (Sigma), whereas rats in the LR and metformin groups received 0.5 g/kg, 2.5 g/kg, and 5 g/kg of LR extract and metformin (150 mg/kg) by oral administration once per day. All animals were given the treatments outlined for 4 wk. At the end of the experimental day, blood was collected from the retro-orbital sinus and centrifuged at 3500 rpm for 10 min at 4 °C. The supernatant was obtained for enzyme-linked immunosorbent assay and metabolomic analysis. The liver, kidney, and pancreas tissues were surgically removed from each rat for H&E staining. The Institutional Animal Care and Use Committee, Inner Mongolian University for Nationalities examined and approved all experimental protocols (Approval No. NM-LL-2021-06-15-1).

High performance liquid chromatography chemical determination
One gram of LR powder was accurately weighed and placed in a 50 mL conical flask. Then 20 mL of methanol solution was added for 30 min ultrasonic extraction, and the liquid was cooled and weighed. Swertiamarin (2 mg), sweroside (1 mg), hesperetin (1 mg), coumarin (4.9 mg), 1.7-dihydroxy-3,8-dimethoxyl xanthone (1 mg), and 1-hydroxy-2,3,5 trimethoxanthone (1 mg) were carefully weighed and put into a 10 mL flask, dissolved in methanol and diluted to scale, shaken well, and filtered through a 0.45 μm microporous filter membrane (each 1 mL contained 0.2 mg swertiamarin, 0.1 mg sweroside, 0.1 mg hesperidin, 0.49 mg coumarin, 0.1 mg 1,7-dihydroxy-3,8-dimethoxone, and 0.1 mg 1-hydroxy-2,3,5-trimethoxone). High performance liquid chromatography (HPLC) analysis was performed on an Agilent 1260 InfinityII HPLC system. ZORBAX SB-C18 5-Micron column (4.6 mm × 250 mm) with mobile phase water (A) - 0.1% phosphate aqueous solution (B) and gradient elution (0-15 min, 30%-35% B; 15 to 25 min, 35%-50% B; 25 to 35 min, 50%-65% B; 35 to 45 min, 65%-70% B; 45 to 50 min, 70%-80% B; 50 to 55 min, 80%-95%; 55-60 min, 95%-100%). The flow rate was set to 1.0 mL/min, the column temperature was set to 30 °C, and the detection wavelength was set at 234 nm. Standards of six compounds were purchased (Sigma-Aldrich, St. Louis, MO, United States), and calibration curves were performed.

Serum biochemical markers analysis

An automated biochemical analyzer (Ichem-340; Icubio, Shenzhen, China) was applied to examine the serum levels of TC, TG, HDL-C, and LDL-C. GLP-1 and insulin were quantified using enzyme-linked immunosorbent assay kits (Bioswamp, Wuhan, China) according to the manufacturer's instructions. Herein, 450 nm was used to measure the absorbance of 100 µL of serum in this experiment.

Oral glucose tolerance test

Rats were fasted for 12 h, after which BG levels were determined using glucometer by obtaining a blood sample from the tail vein at 0 min. Subsequently, the rats were orally administered glucose at 2 g/kg body weight, and BG levels were recorded at 30, 60, 120, and 180 min post-administration. The trapezoidal formula was applied to calculate BG levels to compute the area under the curve (AUC). The value of BG at x minutes was denoted by BG (x), and the AUC was determined using the following formula: oral glucose tolerance test (OGTT) calculation formula: AUC = 0.5 × (BG0 + BG30)/2 + 0.5 × (BG30 + BG60)/2 + 1 × (BG60 + BG120)/2 + 1 × (BG0120 + BG180)/2.

H&E staining

The right liver lobe, kidney tissues, and pancreatic biopsy specimens were embedded with formalin at a concentration of 4% and prepared into paraffin slices measuring 3-5 µm thick. Before being examined under an Olympus microscope equipped with a CCD camera (DS-U3; Nikon, Tokyo, Japan), the tissue sections were stained with H&E. A photographic examination software program (Eclipse E100; Nikon) was utilized for microscopic analysis at × 40 magnification.

Metabolomic analysis of serum samples

The serum samples were analyzed for untargeted metabolite profiles using the XploreMET™ (Metabo-Profile Biotechnology, Shanghai, China). A time-of-flight mass spectrometry system (Pegasus HT; LECO Corp., St. Joseph, MI, United States) was used to assay the component with an Agilent gas chromatograph (GC), and a robotic online derivatization station was used to assay the plasma components. The list of chemicals and reagents used in the metabolomic analysis is reported above. The process of analysis is briefly described in the following parts. Prior to processing, plasma samples were stored at -80 °C. After thawing the samples on ice, a metabolite extraction procedure was conducted. Initially, chloroform was removed from the metabolite extracts using a CentriVap vacuum concentrator. Subsequently, a Free Zone freeze dryer (Labconco, Kansas City, MO, United States) was employed to lyophilize the samples into a dry powder. Fifty milligrams of frozen serum samples were deposited in a microcentrifuge container with 25 mg of zirconium oxide beads and 10 µL of internal calibration standards. For automated homogenization, 50 µL of 50% prechilled methanol was added in each aliquot. After 20 min of centrifugation at 14000 g and 4 °C (Microfuge 20R; Beckman Coulter, Indianapolis, IN, United States), the supernatant was transferred carefully to an autosampler vial (Agilent Technologies, Foster City, CA, United States), dissipated to eliminate chloroform in a CentriVap vacuum concentrator, and then lyophilized utilizing a Free Zone freeze dryer (Labconco). The remaining samples were combined for quality control purposes. The desiccated sample was derivatized with 50 µL of methoxyamine (20 mg/mL in pyridine) at 30 °C for 2 h, then 50 µL of MSTFA (1% TMCS) containing FAMEs as retention indices were added at 37.5 °C for 1 h. The sample derivatization and GC-TOF/MS analysis were conducted with a robotic multipurpose sample with dual heads (Gerstel, Mülheim an der Ruhr, Germany).

After obtaining the raw data, the ChromaTOF software was used to automatically export the original GC-TOF/MS data to XploreMET (Metabo-Profile Biotechnology, Shanghai, China). This enabled programmed baseline denoising and smoothing, peak selection and deconvolution, the creation of a database of references from aggregated quality control samples, metabolite spectrum alignment, missing value rectification and imputation, metabolite verification, and data preprocessing (normalization and standardization). Then, all data was converted into comparable data matrices for statistical analysis. The standard deviation of the experimental measures was scaled and applied to each result, which was then mean-centered. The XploreMET software was used to carry out principal component analysis and orthogonal partial least-square discriminant analysis. The sum of squares of the partial least-squares weights was weighted using the value of the variable importance in the projection. The Kyoto Encyclopedia of Genes and Genomes looked at the metabolic process of many metabolites.

Statistical analysis

The acquired data have been represented as mean ± standard error of the mean. GraphPad prism 7.04 (La Jolla, CA, United States) was used to conduct the statistical analyses. A one-way analysis of variance was applied to the data analysis, and the LSD Multiple Comparison Test was used to evaluate treatment differences. $P < 0.05$ was considered statistically significant, while $P < 0.10$ indicated a trend. Data of differentially expressed metabolites were considered to be statistically significant when a variable was variable importance in the projection ≥ 1.2 and a $P < 0.05$. Univariate statistical analysis (Student's *t*-test) was used to analyze differential metabolites.

RESULTS

HPLC determination of the main compounds of LR

Herein, six bioactive compounds were identified by HPLC analysis as the main bioactive constituents in LR, including swertiamarin, sweroside, hesperetin, coumarin, 1.7-dihydroxy-3,8-dimethoxyl xanthone, and 1-hydroxy-2,3,5 trimethoxanthone with inclusion of 91.10, 6.09, 7.65, 3.04, 29.28, and 3.70 mg/g of dry mater, respectively (Figure 1 and Table 1).

LR protected STZ-induced diabetic rats against the onset of hyperglycemia

As shown in Figure 2A, the body weight was significantly increased in the HFHS diet-fed mice, while STZ injection sharply decreased the body weight in contrast to those in the control group. LR administration and metformin treatment groups significantly reduced ($P < 0.05$ and $P < 0.01$ respectively) the body weight of mice in comparison with those in the model group. In addition, compared with the control group, BG in the model group was significantly increased ($P < 0.01$), whereas LR at 2.5 g/kg and 5 g/kg doses and metformin treatments significantly decreased the serum BG level ($P < 0.05$, Figure 2B). The serum insulin level in the model group was significantly higher ($P < 0.001$) than in the normal group. In comparison to the diabetic model group, LR at 2.5 g/kg and 5 g/kg doses, as well as metformin treatments, significantly ($P < 0.05$) increased serum insulin concentrations (Figure 2C). In contrast, the level of GLP-1 was significantly reduced in the model group by comparison with the control group ($P < 0.001$), but metformin and LR at a dose of 5 g/kg enhanced the serum GLP-1 secretion significantly ($P < 0.05$) as shown in Figure 2D.

The TC and TG levels of the model group were significantly higher ($P < 0.001$) than the control group, whereas serum TC content was significantly lower ($P < 0.05$) in the three LR groups and the metformin group. TG levels were significantly lower in the LR-2.5 and LR-5 groups and the metformin group compared to the diabetic model group (Figure 3A and B). Although the level of HDL-C was not changed in the comparison between the control and model groups, LR treatment at 2.5 g/kg and 5 g/kg doses significantly elevated ($P < 0.05$) the serum concentration of HDL-C compared to the model group (Figure 3C). In terms of serum LDL-C levels, diabetic model animals had significantly higher ($P < 0.05$) LDL-C levels than control animals, whereas the three LR treatment groups and the metformin group had significantly lower ($P < 0.01$) serum LDL-C levels than the model group (Figure 3D).

Effects of LR on histological changes of the pancreas, liver, and kidney tissues in an STZ-induced diabetic rats model

Figure 4 shows that the HFHS diet and STZ-induced diabetic rats had extensive granulation of the β cells and severe vacuolation of the pancreatic islets, while the control rats had normal pancreatic β cells in the islets of Langerhans and the acini. Histological tissue characteristics in the LR and metformin treatment groups showed reduced cell granulation and decreased pancreatic islet vacuolation compared to the diabetic model group. On the other hand, the structure of the liver lobule was complete in the control animals, and cells were organized radially around the central blood vessel. Diabetic model rats had evident macrovesicular steatosis of liver cells. In the LR and metformin groups, the hepatic lobule structure was restored, and the degree of steatosis was significantly lower than in the model group. H&E staining of the kidney sections indicated that the diabetic rats had more visible renal lesions, including glomerular hypertrophy, increased glomerular mesangial cells, and more severe mesangial matrix damage than the control group. The LR and metformin treatments significantly alleviated pathological renal damage in the kidneys of diabetic rats.

Effect of LR on OGTT

In the first 30 min after glucose was given, BG levels were much lower in the LR and metformin groups than in the diabetic group (Figure 5A). Additionally, Figure 5B shows that both LR and metformin treatment significantly improved the AUC values at 30 min, 60 min, 120 min, and 180 min.

Data quality and identification of metabolites

Using untargeted metabolomics, a total of 236 metabolite annotations were determined in the serum samples. Among these, significantly deferentially expressed metabolites mainly include alkaloids and derivatives, lipids and lipid-like molecules, organoheterocyclic compounds, and organic acid and their derivatives. The results of the principal component analysis indicated that the metabolic profiles of the three experimental groups differed significantly, as reflected by the variations between the three sample groups. Furthermore, the quality control sample distances were found to be extremely close, suggesting a high degree of sample data reliability (Figure 6A-C).

Alteration of metabolite levels and biological metabolic pathways

A metabolomic analysis of the differentially expressed metabolites in serum tissues of diabetic rats and the subsequent volcano plot showed that 144 metabolites (67 downregulated and 77 upregulated) were expressed differently between the control and the model group, whereas a comparison of the model and

Table 1 Content of active compounds measured in the *Lomatogonium rotatum* extract

Active compound	Regression equation	R2	Linear range, µg	LR extract, mg/g dry mater
Swertiamarin	Y = 99.413X + 6.3517	1	1.953-9.826	91.10
Sweroside	Y = 198.81X + 0.8476	1	0.431-2.154	6.09
Hesperetin	Y = 437.89X + 0.0685	1	0.119-1.002	7.65
Coumarin	Y = 365.15X + 0.0103	1	0.118-0.590	3.04
1.7-dihydroxy-3.8-dimethoxyxanthone	Y = 214.16X + 2.3704	0.9999	0.098-0.494	29.28
1-hydroxy-2,3,5 trimethoxanthone	Y = 90.498X + 7.1204	0.9999	2.026-10.305	3.70

LR: *Lomatogonium rotatum*.
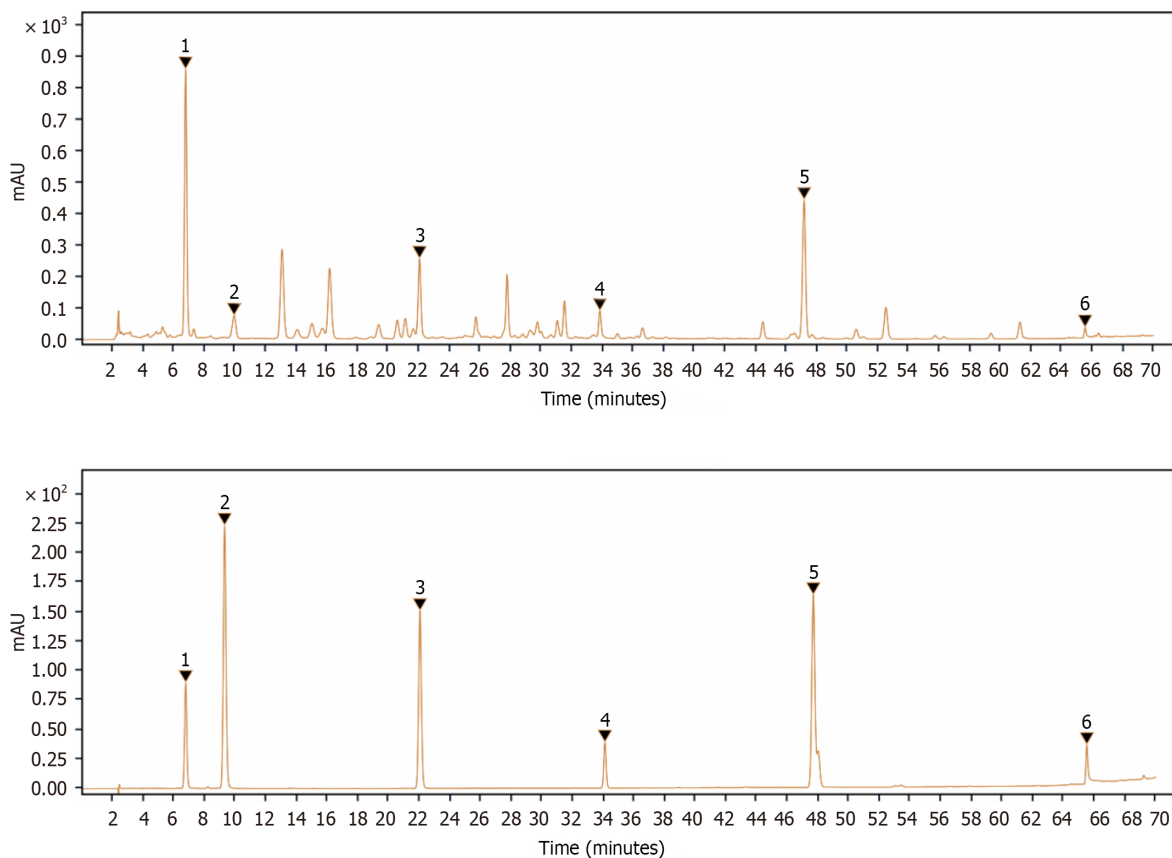
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Figure 1 High performance liquid chromatography determination of the main compounds of *Lomatogonium rotatum*. A: Chromatogram of *Lomatogonium rotatum* samples; B: Chromatogram of the mixture of reference chemicals. 1: Swertiamarin; 2: Sweroside; 3: Hesperetin; 4: Coumarin; 5: 1.7-dihydroxy-3, 8-dimethoxyl xanthone; 6: 1-hydroxy-2,3,5 trimethoxanthone.

LR-5 groups revealed 86 deferentially expressed metabolites (67 downregulated and 19 upregulated) (Figure 7A and B). According to the order of influencing factors in the LR group comparison, the top 13 metabolic pathways were selected, as shown in Figure 7C, including vitamin B6 metabolism and biosynthesis of terpenoids, taurine and hypotaurine metabolism, lipid metabolism scabbard of taurine, selenium metabolism of AA metabolism, pyrimidine, the original generation of bile acid biosynthesis, pantothenic acid salt and histidine biosynthesis and metabolism of coenzyme A, fatty acid, biotin, arginine and proline, and aminoacyl-tRNA biosynthetic pathway. In the LR group, the metabolic pathway of vitamin B6 was the most influential factor, followed by the metabolic pathway of terpenoid backbone biosynthesis, selenium AA, pyrimidine, arginine, and proline.

The representative differential metabolites obtained from the major altered pathways are shown in Table 2. The levels of mevalonic acid-5P, D-proline, L-lysine, taurine, pyridoxal, marshrin, honyucitrin, isoliquiritigenin, 1H-indole-2,3-dione, oxychlordane, phosphorylcholine, Se-adenosylselenohomocysteine, 1-methyladenosine, LysoPE[0:0/18:3(6Z,9Z,12Z)], PE[20:4(8Z,11Z,14Z,17Z)/P-16:0], Bakers

Table 2 Differential metabolites were determined by cross-comparison between different groups in rat serum samples

| No.	Metabolites	Control group *vs* model group		Trend	*P* value	Model group *vs* *Lomatogonium rotatum*		Trend	*P* value
		VIP	FC			VIP	FC		
1	Marshrin	1.46	1.16	Decreased	0.016	1.59	0.68	Increased	0.034
2	Honyucitrin	1.67	1.23	Decreased	0.002	1.54	0.68	Increased	0.047
3	Isoliquiritigenin	1.60	1.75	Decreased	0.004	1.79	0.42	Increased	0.018
4	Pyridoxal	1.53	1.33	Decreased	0.029	1.73	1.47	Increased	0.055
5	1H-indole-2,3-dione	1.21	4.08	Decreased	0.019	1.17	0.34	Increased	0.039
6	Oxychlordane	1.41	1.10	Decreased	0.019	1.30	0.92	Increased	0.039
7	Phosphorylcholine	1.55	1.25	Decreased	0.009	1.26	0.84	Increased	0.045
8	Mevalonic acid-5P	1.32	2.16	Decreased	0.001	1.03	0.56	Increased	0.010
9	D-proline	1.37	1.36	Decreased	0.003	1.80	1.39	Increased	0.019
10	L-lysine	1.40	1.50	Decreased	0.006	1.71	2.42	Increased	0.002
11	Taurine	1.67	1.48	Decreased	0.017	1.61	1.59	Increased	0.006
12	Se-adenosylselenohomocysteine	1.25	1.50	Decreased	0.029	2.24	2.35	Increased	0.001
13	1-methyladenosine	1.47	1.46	Decreased	0.019	1.81	0.58	Increased	0.001
14	LysoPE[0:0/18:3(6Z,9Z,12Z)]	1.64	1.45	Decreased	0.003	1.80	0.63	Increased	0.002
15	PE[20:4(8Z,11Z,14Z,17Z)/P-16:0]	1.80	1.68	Decreased	0.001	1.70	0.72	Increased	0.001
16	Bakers yeast extract	1.51	1.17	Decreased	0.012	1.74	0.70	Increased	0.016
17	Ecgonine methyl ester	1.48	1.10	Decreased	0.013	1.23	0.92	Increased	0.033
18	Dihydrothy	1.66	0.79	Increased	0.013	1.59	0.79	Decreased	0.048
19	Pantothenic acid	1.78	0.65	Increased	0.028	1.97	0.61	Decreased	0.024
20	Aromadendrin 4′-methyl ether 7-rhamnoside	1.35	1.60	Increased	0.030	1.66	2.22	Decreased	0.007

The significance of potential metabolites in the serum of rats induced by *Lomatogonium rotatum* is shown in this table. FC: Fold change; VIP: Variable importance in projection.

yeast extract, and ecgonine methyl ester showed a significant decrease in the model group in comparison with the control group. By contrast, LR treatment dramatically increased the above metabolites in the serum samples. Moreover, the levels of dihydrothy, pantothenic acid, and aromadendrin 4′-methyl ether 7-rhamnoside were greatly elevated in the model group than in the control group. Nevertheless, LR obviously reduced the levels of these metabolites. The results indicated that most of the metabolites were reversed by LR extract treatment and were regulated to return to levels that were comparable to those of the control group.

DISCUSSION

Obesity-related disorders, specifically T2DM, have become one of the world's greatest health concerns. According to multiple studies, a disturbance in energy metabolism is the primary risk factor for the development of T2DM. Current clinical applications have recommended single-target medications; however, overcoming the problems with these drugs has been difficult. As a result, traditional medicines with the advantages of multitargets and multimechanisms could be potential treatments for T2DM. LR is a bitter medicinal herb in traditional Mongolian medicine used for bodyweight reduction. However, the pharmacological effects of LR and its specific metabolic changes on T2DM are not entirely understood. Biological cells respond to a disease state by changing the concentration of a large number of metabolites to maintain homeostasis[18]. In this study, serum metabolic profiles were generated using ultra-HPLC, and the potential mechanisms of LR in T2DM were examined.

Herein, the HFHS diets plus STZ is a relatively stable method for modeling T2DM. According to the results, the HFHS-induced rats had increased body weight and considerably elevated TC, TG, and LDL-
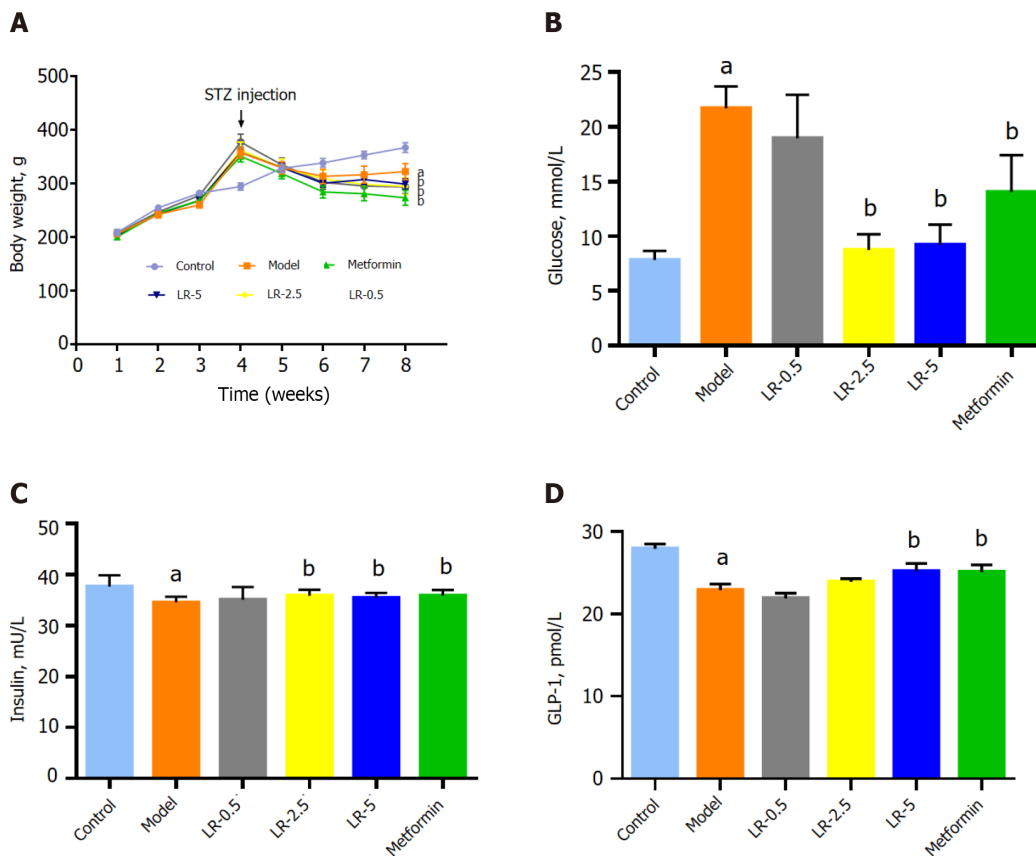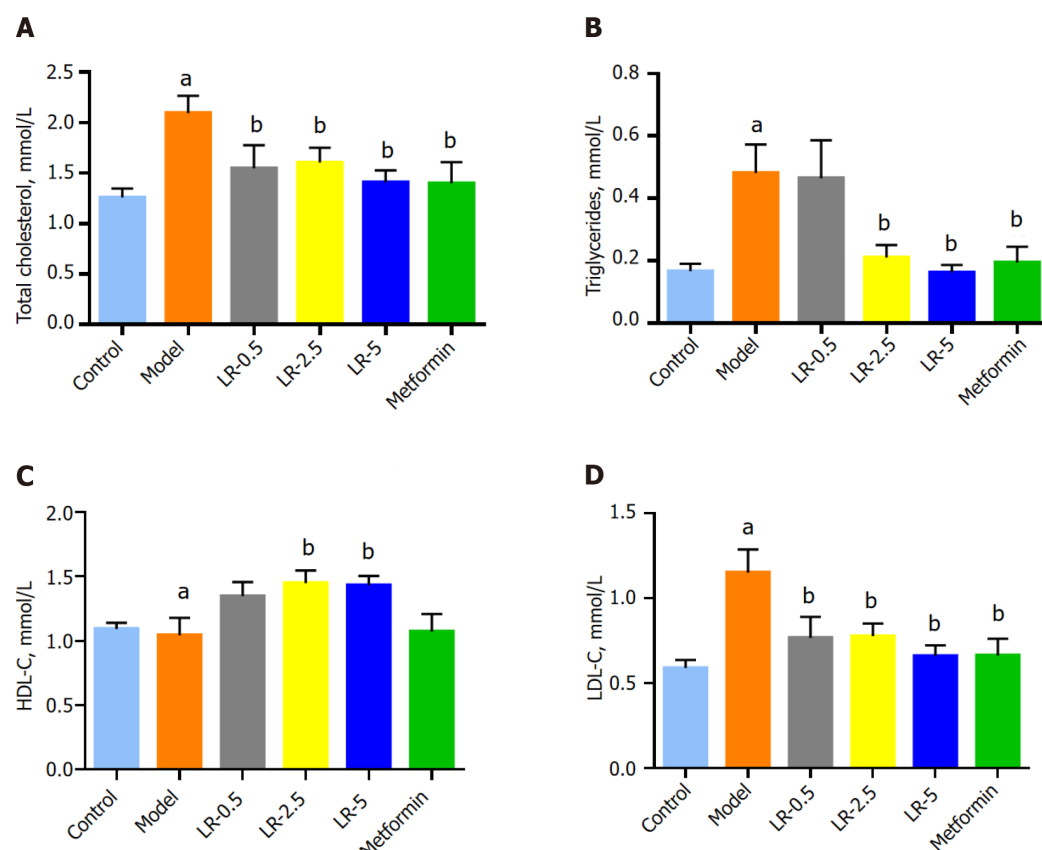
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Figure 2 Effects of *Lomatogonium rotatum* on body weight, serum glucose, insulin, and glucagon-like peptide 1 levels in the diabetic rat model. A: Body weight; B: Serum glucose; C: Insulin; D: Glucagon-like peptide 1. The data represent means ± standard error of the mean (*n* = 10). [a]*P* < 0.05 *vs* the control group; [b]*P* < 0.05 *vs* the model group. GLP-1: Glucagon-like peptide 1; LR: *Lomatogonium rotatum*; STZ: Streptozotocin.

C plasma levels compared with normal rats. Additionally, increased glucose tolerance significantly impaired the plasma levels of glucose, GLP-1, and insulin. This was confirmed that an HFHS diet causes weight increase, insulin sensitivity, and impaired glucose tolerance[19]. Therefore, the HFHS-induced animal model indicated a typical obesity phenotype.

According to an HPLC analysis, six main compounds were identified from the LR extract. The most abundant components were swertiamarin, hesperetin, and coumarin, which have been previously documented with effects on obesity and hyperglycemia[20-22], making them the likely effectors of the pharmacological activities of the LR extract. The presence of xanthone, another key component in the LR extract, is known to have numerous pharmacological effects, including anti-inflammatory and antimycobacterial properties[23]. Nevertheless, its hypoglycemic action has yet to be investigated. In addition, experimental results showed that high doses and a medium dose of LR administration indicated similar outcomes as metformin. The LR administration significantly reduced the body weight in the model group and showed lower serum glucose and lipid contents. Several studies have reported that abnormalities of lipid contents in serum are highly related to hyperglycemia[24]. In this study, the levels of TC, TG, HDL-C, and LDL-C were significantly reversed after LR administration. TC, TG, LDL-C, and HDL-C are important biomarkers, which indicate hyperlipidemia. Moreover, lipid abnormalities drive the increase in lipid deposition[25]. Our findings indicated that LR may have a potent hypolipidemic effect by decreasing plasma levels of TC, TG, and LDL-C while elevating HDL-C levels. LR could have a positive effect on the control of hyperlipidemia.

Dyslipidemia is caused in part by a correlation between carbohydrate and lipid metabolism and aberrant BG levels[26]. Herein, glucose levels were significantly elevated in the serum, and the OGTT results indicated that diabetic rats developed impaired glucose tolerance. Regarding glucose metabolism, LR treatment lowered BG and greatly improved glucose tolerance. The reduction in BG by LR administration was associated with a significant improvement in glucose intolerance, as revealed by the decreased AUC value in the OGTT response. OGTT was usually used to assess peripheral insulin action and insulin resistance *in vivo*. The OGTT results were accompanied by insulin levels in serum. Insulin resistance is related to T2DM and is characterized by the decreased response of insulin-sensitive cells or tissues. It can cause impaired peripheral glucose consumption and develop hyperglycemia and compensatory hyperinsulinemia. Moreover, the plasma GLP-1 level was improved by LR treatment. GLP-1 is a hormone primarily produced in the L cells of the distal ileum and colon. It promotes insulin

Figure 3 Effects of *Lomatogonium rotatum* on serum total cholesterol, triglycerides, high-density lipoprotein-cholesterol, and low-density lipoprotein cholesterol levels in diabetic rats. A: Total cholesterol; B: Triglycerides; C: High-density lipoprotein-cholesterol; D: Low-density lipoprotein cholesterol. The data represent means ± standard error of the mean (*n* = 10). [a]$P < 0.01$ and [b]$P < 0.05$ *vs* the model group. HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein cholesterol; LR: *Lomatogonium rotatum*.
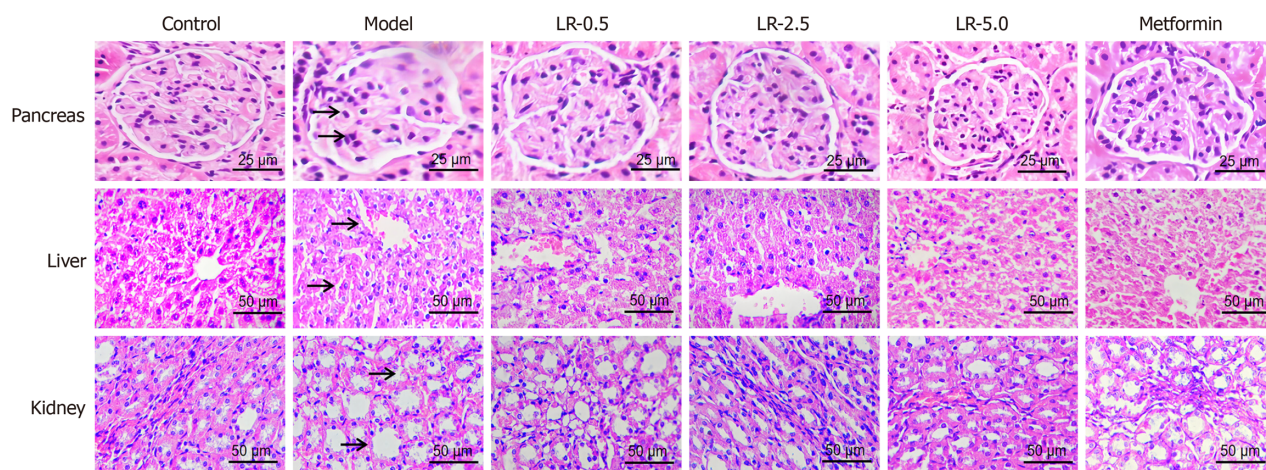
Figure 4 Effects of *Lomatogonium rotatum* on histological changes of the pancreas, liver, and kidney tissues in the streptozotocin-induced diabetic rat model. Arrows indicate β-cell vacuolation and granulation in the pancreas, impaired central vein and steatosis in the liver, and renal lesions and glomerular hypertrophy in the kidney. LR: *Lomatogonium rotatum*.

secretion while inhibiting glucagon synthesis. It also plays a significant role in glucose homeostasis and is a key biomarker of abnormalities in glucose metabolism[27]. The exposure of cultured gut endocrine cells to bitter substances stimulates the release of hormones, including GLP-1[28]. Therefore, LR administration significantly improved insulin sensitivity and GLP-1 secretion in diabetic rats. Taken together, the physiological results expressively revealed that LR administration had the effect of reducing obesity and improving lipid and carbohydrate metabolism.
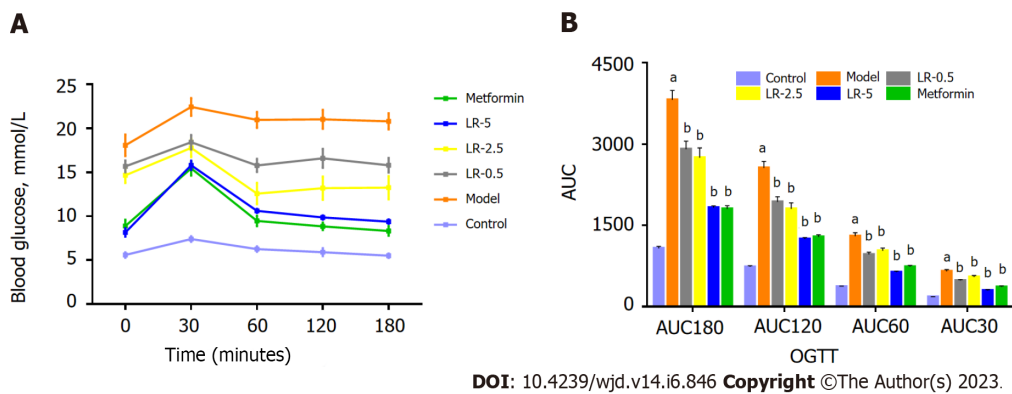
Figure 5 Effects of *Lomatogonium rotatum* on the oral glucose tolerance test of diabetic rats. A: Changes in blood glucose from 0 to 180 min; B: Values for the area under the curve. The data represents means ± standard error of the mean (*n* = 10). [a]*P* < 0.05 *vs* the control group; [b]*P* < 0.05 *vs* the model group. AUC: Area under the curve; LR: *Lomatogonium rotatum*; OGTT: Oral glucose tolerance test.
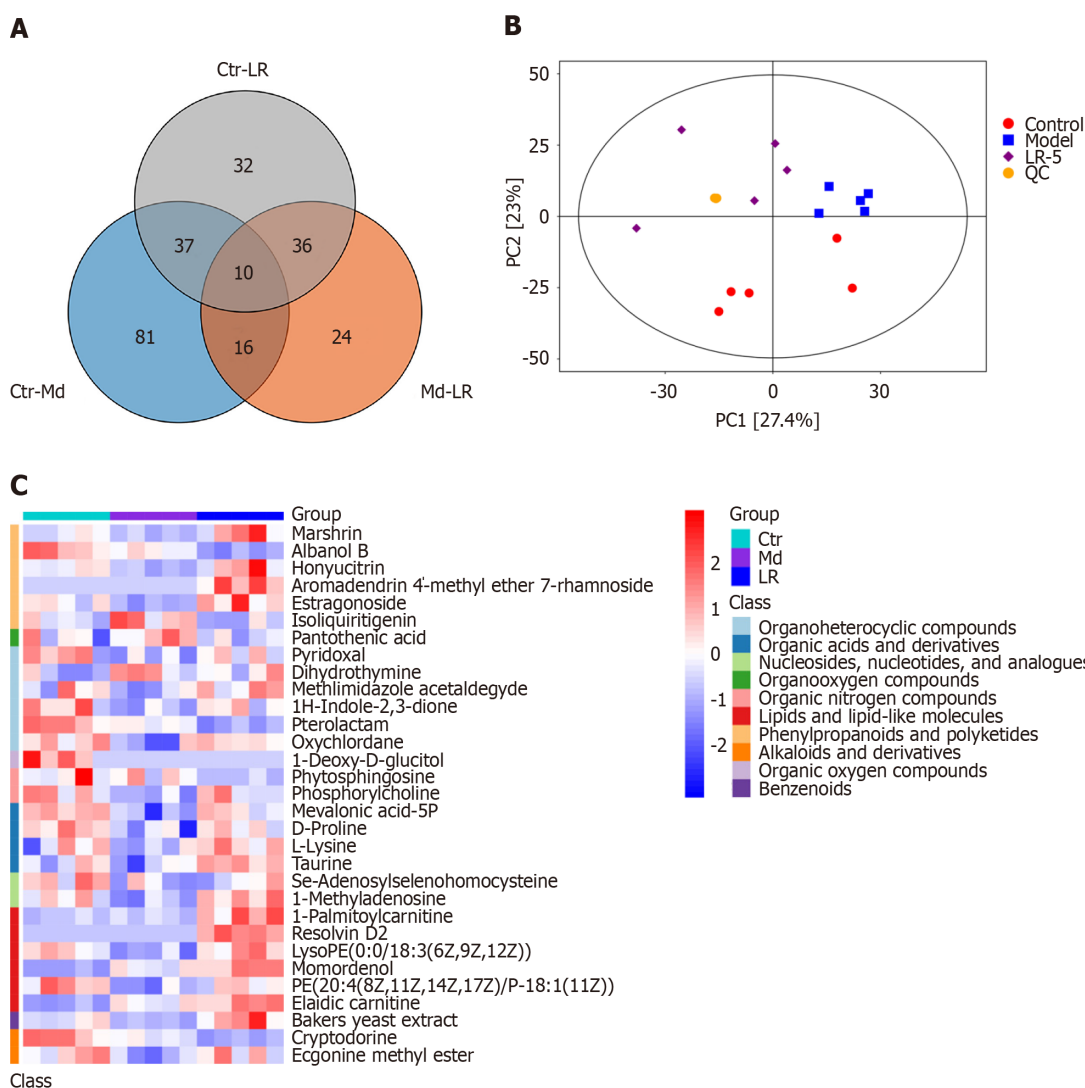
Figure 6 Metabolomic analysis of *Lomatogonium rotatum*-treated streptozotocin-induced diabetic rats. A: The Venn diagram displays the amount of metabolites with differential expression. Different colors indicate distinct comparisons, whereas overlapping regions show differentially expressed metabolites shared by two groups; B: Serum metabolic characteristics of different groups were determined by a principal component analysis diagram; C: Cluster heat map of differentially-expressed metabolites in three experimental groups. In each sample, red and blue colors indicated higher and lower expression, respectively. LR: *Lomatogonium rotatum*; QC: Quality control.

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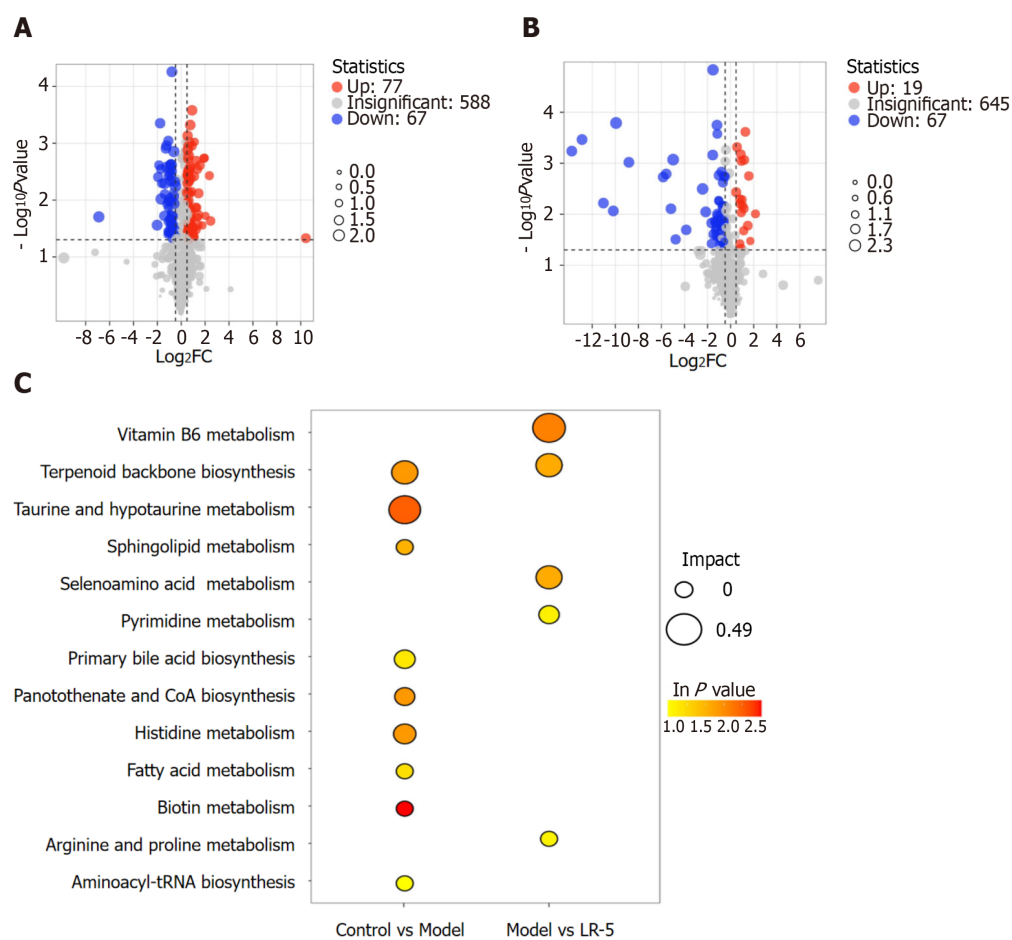
Figure 7 Differential metabolites and pathways across groups. A and B: A volcano diagram illustrated the distinct metabolite compositions between control *vs* model (A) and model *vs* LR-5 (B). Green and red colors represent significant upregulation and downregulation of metabolites, respectively. Significant deferentially expressed metabolites were determined based on a *P* value < 0.05 and a log2fold-change of at least 2.0; C: XploreMET (Metabo-Profile) was used to evaluate the Kyoto Encyclopedia of Genes and Genomes metabolic pathways of the differential metabolites. FC: Fold change; LR: *Lomatogonium rotatum*; VIP: Variable importance in projection.

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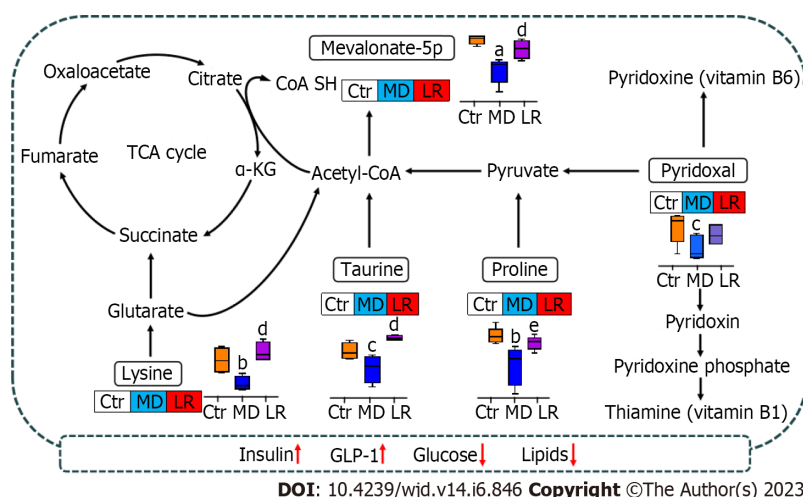
Figure 8 Schematic summary of metabolic pathways related to the *Lomatogonium rotatum* effect on streptozotocin-induced diabetic rats. The relative levels of significantly altered metabolites were presented in different colors. The blue rectangle represents downregulation, the red rectangle represents upregulation, and the gray rectangle reveals no change in contrast to the control. [a]*P* < 0.001, [b]*P* < 0.01 and [c]*P* < 0.05 *vs* the control group; [d]*P* < 0.01 and [e]*P* < 0.05 *vs* the model group. Ctr: Control group; LR: *Lomatogonium rotatum*-treated group; MD: Model group; TCA: Tricarboxylic acid.

Metabolomics is a high-throughput technology that has been widely used for identifying biomarkers, revealing metabolic pathways, and unraveling the mechanisms of metabolic diseases[29]. In this study, untargeted metabolomics technology was used to analyze serum metabolites and the metabolic pathways of LR administration and to explore its mechanism of lowering BG and anti-diabetic action. Our findings revealed that the metabolic pathway of vitamin B6 was the most influential factor, followed by terpenoid backbone biosynthesis, selenium AA, pyrimidine, arginine, and proline. Metabolites such as pyridoxal, mevalonic acid-5P, proline, lysine, and taurine have been well reported on the regulation of T2DM, dyslipidemia, inflammation, and oxidative stress[30-32]. In addition, LR administration promoted energy metabolism related to AA.

Recent studies reported AAs may be potentially important in the prevention of diabetes and diabetes-associated complications[33]. Protein and glucose metabolism are strongly interconnected and consequently regulated at the metabolic and molecular levels. AAs relate to glucose metabolism *via* gluconeogenesis, which is a catabolic breakdown of AAs. In metabolomics studies, two important potential biomarkers, *i.e.* D-proline and L-lysine, were identified.

Lysine supplements decreased diabetic complications linked with T2DM in the diabetic rat models and *in vitro*[34,35]. Lysine is an essential AA that plays a major role in calcium absorption, building muscle protein, and the body's production of hormones, enzymes, and antibodies. Animal and human studies have shown that it has also demonstrated various beneficial effects in the treatment/prevention of diabetes and/or its complications. In diabetes-induced animal models, lysine has shown beneficial effects in lowering BG as well as acting as an inhibitor of protein glycation[36]. Lysine is known to react with glucose, with the glycated AA being excreted in the urine, and it has been shown to markedly minimize the glucose response to dietary carbohydrates without influence on insulin response[37]. Lysine could be catabolized to participate in energy metabolism. One mechanism involves the conversion of lysine to glutaryl-CoA, which is then converted to acetyl-CoA[38]. In the tricarboxylic acid cycle, lysine is metabolized to 2-ketoglutaric acid, which then forms succinate. Additionally, proline accelerates insulin secretion in both clonal β cells and isolated mouse islets[39,40].

In the current study, the elevated level of insulin in the LR group could be influenced by the high proline level. Moreover, proline could be converted to glutamate and metabolized to pyruvate, which is a key metabolite joining the tricarboxylic acid cycle[41]. Pyruvate metabolized to acetyl-CoA participates in the regulation of energy metabolism. Subsequently, the inappropriate glucogenic metabolism caused by the HFHS diet could be recovered by LR administration (Figure 8). In this view, LR administration has the potential to elevate lysine, and proline levels may help with diabetes management and blood sugar control.

Vitamin B metabolism was modified after LR administration, and the level of pyridoxal, a key metabolite, was restored in the LR groups. Vitamin B6 is an essential cofactor in various transamination, decarboxylation, glycogen hydrolysis, and synthesis pathways involving carbohydrate, sphingolipid, AA, heme, and neurotransmitter metabolism. The active form of vitamin B6, *i.e.* 5'-pyridoxine phosphate, is associated with protecting cells from DNA damage. 5'-pyridoxine phosphate acts as a coenzyme in about 160 enzymatic reactions, regulating the metabolism of glucose, lipids, AAs, heme, DNA/RNA, and many neurotransmitters[42]. Furthermore, the effect of vitamin B supplementation in preventing diabetic microvascular complications has long been the subject of study. Studies of vitamin B6 (pyridoxine, pyridoxine 50-phosphate) and high-dose vitamin B1 have shown that proteinuria can be inhibited in diabetic animal models[43]. In patients with T2DM and nephropathy, the combination of vitamin B1 (thiamine) and vitamin B6 (pyridoxine) significantly reduced the glycosylation of leukocyte nuclear DNA[44]. Addressing the vitamin B deficiency associated with diabetes that has been seen in experimental diabetes, particularly in tissues where vascular problems develop, may help to achieve the therapeutic advantage of vitamin B supplementation[45,46].

CONCLUSION

In this study, an HPLC method was used to identify swertiamarin, sweroside, hesperetin, coumarin, 1.7-dihydroxy-3,8-dimethoxyl xanthone, and 1-hydroxy-2,3,5 trimethoxanthone as the main chemical constituents of LR. Administration of LR extract for 4 wk in T2DM rats resulted in improvement in BG, glucose tolerance, TC, TG, and LDL-C, restoration of insulin and GLP-1 activity, and improvement in the histological properties of tissues and organs. The results suggested that the hypoglycemic effect of LR may be associated with alterations in serum metabolites, which in turn may facilitate insulin and GLP-1 activities, leading to a reduction in BG and lipid profiles.

ARTICLE HIGHLIGHTS

Research background

Although *Lomatogonium rotatum* (LR) has a long history of usage as a hypoglycemic agent in Mongolian

folk medicine, the evidence-based pharmacological properties and mechanisms of action of this medicinal plant have not yet been thoroughly explained.

Research motivation

The current study explored the hypoglycemic effects and mechanism of LR in a high-fat, high-sugar diet and streptozotocin-induced type 2 diabetic rat model.

Research objectives

The current study aimed to emphasize the hypoglycemic action mechanism of LR in a type 2 diabetic rat model and examine potential biomarkers to obtain mechanistic insight into the serum metabolite modifications.

Research methods

A combination of feeding a high-fat, high-sugar diet and streptozotocin injections were applied to develop type 2 diabetes in rats. The high performance liquid chromatography technique was used to determine the chemical composition of LR. LR extract was given through oral gavage at doses of 0.5 g/kg, 2.5 g/kg, and 5 g/kg on a weekly basis for a period of 4 wk. The histopathological examination, as well as the assessment of blood glucose, insulin, glucagon-like peptide 1 (GLP-1), and lipid levels, were used to evaluate the anti-diabetic effects of LR extract. A method known as untargeted metabolomics was used in order to study the metabolites found in serum.

Research results

The primary active components found in LR included swertiamarin, sweroside, hesperetin, coumarin, 1.7-dihydroxy-3,8-dimethoxyl xanthone, and 1-hydroxy-2,3,5 trimethoxanone. When compared to the model group, the LR therapy resulted in a large increase in plasma insulin and GLP-1 levels while simultaneously resulting in a significant reduction in blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, and an oral glucose tolerance test. Analysis of blood samples using an untargeted metabolomic approach found a total of 236 metabolites, of which 86 showed altered levels of expression in the model compared to the LR group. In addition, LR caused significant changes in the levels of metabolites such as vitamin B6, mevalonate-5P, D-proline, L-lysine, and taurine. These metabolites are involved in the regulation of the metabolic pathways for vitamin B6, selenium amino acids, pyrimidine, arginine, and proline.

Research conclusions

These findings indicated that the hypoglycemic effect of LR may be associated with alterations in serum metabolites, which in turn may facilitate insulin and GLP-1 activities, leading to a reduction in blood glucose and lipid profiles.

Research perspectives

Further research is required to confirm the levels of target gene or protein expression that are linked to the changed metabolic pathways and to demonstrate how LR extract lowers blood glucose at the molecular level.

FOOTNOTES

ORCID number: Li-Li Dai 0000-0003-4821-596X; Sung-Bo Cho 0000-0002-2593-2758; Li-Sha A 0000-0002-0137-1677; Laxinamujila Bai 0000-0001-6445-3701; Gen-Na Ba 0000-0002-0834-3863; Ming-Hai Fu 0000-0002-5096-8744.

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ORIGINAL ARTICLE

Basic Study

Alteration of intestinal microbiota is associated with diabetic retinopathy and its severity: Samples collected from southeast coast Chinese

Xue-Mei Gu, Chao-Yin Lu, Jian Pan, Jian-Zhong Ye, Qi-Han Zhu

Xue-Mei Gu, Qi-Han Zhu, Department of Endocrinology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

Xue-Mei Gu, Qi-Han Zhu, Wenzhou Key Laboratory of Diabetes Research, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

Chao-Yin Lu, Department of Endocrinology, Taizhou Central Hospital (Taizhou University Hospital), Taizhou 318000, Zhejiang Province, China

Jian Pan, Department of Ophthalmology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

Jian-Zhong Ye, Department of Clinical Laboratory, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

Corresponding author: Qi-Han Zhu, MM, Attending Doctor, Department of Endocrinology, The First Affiliated Hospital of Wenzhou Medical University, The South of Shangcai Village, Nanbaixiang Town, Ouhai District, Wenzhou 325000, Zhejiang Province, China. dr_zhuqihan@163.com

Abstract

BACKGROUND
Current approaches for the therapy of diabetic retinopathy (DR), which was one of leading causes of visual impairment, have their limitations. Animal experiments revealed that restructuring of intestinal microbiota can prevent retinopathy.

AIM
To explore the relationship between intestinal microbiota and DR among patients in the southeast coast of China, and provide clues for novel ways to prevention and treatment methods of DR.

METHODS
The fecal samples of non-diabetics (Group C, *n* = 15) and diabetics (Group DM, *n* = 30), including 15 samples with DR (Group DR) and 15 samples without DR (Group D), were analyzed by 16S rRNA sequencing. Intestinal microbiota

compositions were compared between Group C and Group DM, Group DR and Group D, as well as patients with proliferative diabetic retinopathy (PDR) (Group PDR, *n* = 8) and patients without PDR (Group NPDR, *n* = 7). Spearman correlation analyses were performed to explore the associations between intestinal microbiota and clinical indicators.

RESULTS
The alpha and beta diversity did not differ significantly between Group DR and Group D as well as Group PDR and Group NPDR. At the family level, *Fusobacteriaceae*, *Desulfovibrionaceae* and *Pseudomonadaceae* were significantly increased in Group DR than in Group D ($P < 0.05$, respectively). At the genera level, *Fusobacterium*, *Pseudomonas*, and *Adlercreutzia* were increased in Group DR than Group D while *Senegalimassilia* was decreased ($P < 0.05$, respectively). *Pseudomonas* was negatively correlated with NK cell count ($r = -0.39$, $P = 0.03$). Further, the abundance of genera *Eubacterium* ($P < 0.01$), *Peptococcus*, *Desulfovibrio*, *Acetanaerobacterium* and *Negativibacillus* ($P < 0.05$, respectively) were higher in Group PDR compared to Group NPDR, while *Pseudomonas*, *Alloprevotella* and *Tyzzerella* ($P < 0.05$, respectively) were lower. *Acetanaerobacterium* and *Desulfovibrio* were positively correlated with fasting insulin ($r = 0.53$ and 0.61, respectively, $P < 0.05$), when *Negativibacillus* was negatively correlated with B cell count ($r = -0.67$, $P < 0.01$).

CONCLUSION
Our findings indicated that the alteration of gut microbiota was associated with DR and its severity among patients in the southeast coast of China, probably by multiple mechanisms such as producing short-chain fatty acids, influencing permeability of blood vessels, affecting levels of vascular cell adhesion molecule-1, hypoxia-inducible factor-1, B cell and insulin. Modulating gut microbiota composition might be a novel strategy for prevention of DR, particularly PDR in population above.

Core Tip: Current approaches for the therapy of diabetic retinopathy (DR) have their limitations. Our study revealed that alteration of gut microbiota was associated with DR and its progression, and further, this association was mediated by multiple mechanisms including producing short-chain fatty acids, influencing permeability of blood vessels, affecting levels of vascular cell adhesion molecule-1, hypoxia-inducible factor-1, B cell and insulin. Hence, reconstruction of gut microbiota might be a promising strategy for prevention of DR.

INTRODUCTION
For the moment, diabetes is one of the fastest developing and worldwide metabolic diseases, with multiple complications such as diabetic retinopathy (DR). Global pool analysis of DR in 2010 revealed the proportion of DR, and vision-threatening DR in diabetics was 34.6%, 10.2% respectively[1]. DR will cause visual impairment and even blindness in adults aged 20 to 74 years old, and is considered as one of the primary causes[2]. DR is subdivided into non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). PDR is less common in patients but more threatening to vision compared with NPDR[3]. The treatment of PDR and visually threatening diabetic macular edema (DME) is a main research topic on DR. Laser therapy, anti-angiogenic therapy, anti-inflammatory therapy and surgery are major treatments for PDR. Laser therapy is a classic tool for severe NPDR and PDR, aiming to preserve visual acuity[4]. However, laser would impair the central vision and night vision[5]. As a main anti-angiogenic therapy, anti-vascular endothelial growth factor (VEGF) is a recommended therapy of DR refractory to laser treatment and DME. Contrast to laser monotherapy, anti-VEGF therapy can improve visual acuity in inpatients with DME or PDR[6,7]. However, considering limited half-life time of anti-VEGF agents, the repetitive injections of anti-VEGF are

required at one or two months intervals, causing increased financial burden, increased occurrence of endophthalmitis and elevated intraocular pressure[8]. Besides, the long-term therapy of anti-VEGF would reduce patient compliance, increase the incidence of treatment interruption, and result in deterioration finally[9]. Almost nearly 50% patients are insensitive or even non-responsive to anti-VEGF therapy, but new approved anti-angiogenic therapies as well as effective and evidence-based replacement treatments are absent[10,11]. Although glucocorticoids can be used in patients who failed to respond to anti-VEGF, the role in treating PDR still need further confirmation and the side effect of elevating blood glucose constrains its application in diabetics[9]. Other treatments including anti-protein kinase C, angiotensin receptor blockers, fenofibrate have their own flaws[12-16].

Chronic low-grade inflammations are already recognized as pivotal players in the development of diabetes and its complications including DR. Besides the anti-inflammatory effect *via* generating short chain fatty acid such as butyrate[17], intestinal microbiota also plays a pro-inflammatory role by increasing intestinal permeability, releasing lipopolysaccharide (LPS) which was relevant with distant inflammatory response and impacted cytokines such as TNF-α and IL-6[18,19]. The roles of microbiota on inflammation may explain its possible contribution on occurrence and development of DR. An animal experiments showed that intermittent fasting can prevent the occurrence of DR[10]. Only four studies aiming at the relationship between human intestinal microbiota and DR have been found[20-23]. Jayasudha *et al*[21] performed Illumina sequencing of the internal transcribed spacer 2 region which mainly detects fungus. Three other studies performed 16S rRNA sequencing to distinguish the microbiota between diabetics with DR and without DR[20,22,23]. Moubayed *et al*[20] only analyzed fecal genus Bacteroides among healthy volunteers, diabetic patients with DR and without DR, lack of analysis of the other microbial community types. As for the other two studiers, one of their limits was that diabetics enrolled are always treated with metformin[22,23]. Metformin can reduce the severity of DR and incidence of NPDR independently and the mechanisms might be anti-angiogenesis and anti-inflammation[24-27]. Notwithstanding no studies have demonstrated that intestinal microbiota involves in the effect of metformin on DR, metformin should also be considered as a confounding factor which may affect the accuracy of the conclusion about relationship between intestinal microbiota and DR stated by Huang *et al*[22]. Moreover, the effect of metformin on different microbiota were inconsistent in type 2 diabetes mellitus (T2DM) patients, which possibly be impacted by duration of diabetes, gender and race[28]. Thus, metformin taken by whole T2DM subjects still probably complicate the analysis of gut microbiota[24]. The abundance of intestinal microbiota was obviously affected by diet and geographic proximity[29,30]. Different intestinal microbiota may be relevant to same diseases among different persons from different areas and with different dietary habits. Our study is focus on exploring the differential bacteria between diabetic patients with DR and without DR, as well as diabetic patients with PDR and NPDR in south Zhejiang and north Fujian in China, aiming to unravel the link between intestinal microflora and DR, and find a new therapeutic target for DR, especially PDR.

MATERIALS AND METHODS

Study population and sample collection

For this study, 45 samples were obtained from patients who are hospitalized in the department of endocrinology in the 1st Affiliated Hospital of Wenzhou medical university from August, 2018 to September, 2020. Patients were divided into non-diabetics (Group C, *n* = 15) and T2DM patients (Group DM, *n* = 30), which was further divided into patients with DR (Group DR, *n* = 15) and patients without DR (Group D, *n* = 15). Further, Group DR was divided into patients with PDR (Group PDR, *n* = 8) and patients without PDR (Group NPDR, *n* = 7). The enrolled patients are aged between 30-80 years old without conditions as pregnant, lactation, current smoker, current drinker, BMI ≥ 27, prescribed for metformin, alpha glycosidase inhibitor, antibiotics, probiotics, glucocorticoids, cathartics or PPI within 3 mo, rheumatoid arthritis, inflammatory bowel disease, or gastrointestinal tract operation. After admitted into our department, demographic, medical history, physical examination data were collected and several biochemical tests were performed. Participants self-collected a fecal sample, which were collected by patients, and stored at -80 ℃ later in less than 24 h. This study was approved by the Ethics Committee of the 1st Affiliated Hospital of Wenzhou medical university. All participants gave their informed consent. The trial register number is 2018-129.

DNA extraction and amplification

Fecal samples were snap frozen and stored at -80 ℃ after collection. Bacterial DNA was isolated from the fecal samples using MagPure Soil DNA LQ Kit (Magen, United States) following the manufacturer's instructions. DNA concentration and integrity were measured by Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) and agarose gel electrophoresis, respectively. Polymerase chain reaction (PCR) amplification of the V3-V4 hypervariable regions of the bacterial 16S rRNA gene was carried out in a 25 µL reaction using universal primer pairs (343F: 5'-TACGGRAG-GCAGCAG-3'; 798R: 5'-AGGGTATCTAATCCT-3'). The reverse primer contained a sample barcode and both primers were connected with an Illumina sequencing adapter.

Library construction and sequencing

The amplicon quality was visualized using gel electrophoresis. The PCR products were purified with Agencourt AMPure XP beads (Beckman Coulter Co., United States) and quantified using Qubit dsDNA assay kit. The concentrations were then adjusted for sequencing. 16S rDNA sequencing were performed using Illumina MiSeq platform at Shanghai OE Biotech Co., Ltd.

Bioinformatic analysis

Paired-end reads were preprocessed using Trimmomatic software to detect and cut off ambiguous bases(N)[31]. It also cut off low quality sequences with average quality score below 20 using sliding window trimming approach. After trimming, paired-end reads were assembled using FLASH software [32]. Parameters of assembly were: 10 bp of minimal overlapping, 200 bp of maximum overlapping and 20% of maximum mismatch rate. Sequences were performed further denoising as follows: Reads with ambiguous, homologous sequences or below 200 bp were abandoned. Reads with 75% of bases above Q20 were retained using QIIME software (version 1.8.0)[33]. Then, reads with chimera were detected and removed using VSEARCH[34]. Clean reads were subjected to primer sequences removal and clustering to generate operational taxonomic units (OTUs) using VSEARCH software with 97% similarity cutoff[34]. The representative read of each OTU was selected using QIIME package. All representative reads were annotated and blasted against Silva database (Version 123) using Ribosomal Database Project classifier (confidence threshold was 70%)[35]. The microbial diversity in fecal samples was estimated using the alpha diversity that include Chao1 index, Shannon index and Simpson index. The Unifrac distance matrix performed by QIIME software was used for weighted Unifrac principal coordinates analysis (PCoA) construction. The 16S rRNA gene amplicon sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China).

Statistical analysis

Data with normal distribution and homogeneity of variance were compared using independant samples *t* test, otherwise, were compared using Wilcoxon test. Comparisons between groups were performed with the clinical characteristics. For associations between clinical characteristics and gut microbial, Spearman correlation analysis were performed using R version 3.6.1. Correction for multiple testing was performed using false discovery rate with the Benjamini–Hochberg. False discovery rate values < 0.05 were considered statistically significant.

RESULTS

Clinical and biochemical characteristics

The clinical and biochemical characteristics were compared between Group DM and C as well as Group D and DR, Group PDR and NPDR (Tables 1 and 2). The age, sex proportion and BMI did not differ between the three pairs mentioned above. Further, the other indexes were comparable between Group DM and C except for fasting blood glucose, glycated hemoglobin A1c (Tables 1 and 2). Compared to Group D, neutrophil to lymphocyte ratio, CD4$^+$ T cell count were significantly increased in Group DR (P < 0.05, P < 0.05), whereas, B cell count, CD8$^+$ T cell count, NK cell count, percentage of NK cell were decreased (P < 0.01, P < 0.05, P < 0.01, P < 0.05, Table 2). Patients in Group PDR had a significantly lower level of estimated glomerular filtration rate (eGFR) and B cell count (P < 0.05, P < 0.01), and a higher level of fasting insulin compared with NPDR (P < 0.05, Table 2).

Sequencing summary

Total 890469 sequences read with an average of 19788.2 reads per sample were obtained among the 45 samples. 46551 OTUs were observed totally, with a mean of 1034.467 OTUs. The phyla *Bacteroidete* was the dominant intestinal microbiota with approximately 40% (Figure 1). The other three phyla dominated in microbiota were *Firmicutes*, *Proteobacteria* and *Actinobacteria*, with average relative abundances of 29.1%, 19.6% and 5.2% respectively (Figure 1).

Fecal microbiota diversity

The Chao1, Shannon, simpson indexes were significantly higher in Group C compared with Group DM (P < 0.001, P < 0.001, P < 0.001, Figure 2A-C). However, they did not differ significantly between Group D and Group DR as well as between Group PDR and Group NPDR (P > 0.05, Figure 2D-I).

Weighted and unweighted PCoA showed a distinct distance between Group C and Group DM (Adonis, P < 0.01, P < 0.01, Figure 3A and B), whereas Group D and Group DR had no distinction (P > 0.05, P > 0.05, P > 0.05, P > 0.05, Figure 3C and D). However, the microbiota community in Group PDR was not differed from Group NPDR (P > 0.05, P > 0.05, Figure 3E and F).

Table 1 Basic characteristics compared between Group DM and Group C

Characteristic	Group DM *vs* Group C		
	Group DM (*n* = 30)	Group C (*n* = 15)	*P* value
Age	55.93 ± 9.58	59.73 ± 16.46	NS
Gender, male/female	19/11	9/6	NS
Height	165.70 ± 8.07	162.20 ± 7.78	NS
Weight	64.75 ± 7.67	62.53 ± 8.29	NS
Body mass index	23.47 ± 2.21	23.73 ± 2.27	NS
Waist circumference	84.75 ± 7.78	84.67 ± 8.36	NS
Hip circumference	91.42 ± 7.32	92.07 ± 7.29	NS
Waist hip ratio	0.93 ± 0.05	0.92 ± 0.08	NS
Waist height ratio	0.51 ± 0.05	0.52 ± 0.05	NS
Systolic blood pressure	128.43 ± 11.98	127.80 ± 14.03	NS
Fasting glucose	8.29 ± 2.79	5.34 ± 0.95	< 0.01
HbA1c	9.94 ± 1.96	5.63 ± 0.31	< 0.01
Triglycerides	1.81 ± 1.82	1.72 ± 0.77	NS
LDL-C	3.00 ± 0.97	2.94 ± 0.88	NS
eGFR	92.29 ± 27.65	87.21 ± 30.00	NS

Data are presented as mean ± SD. HbA1c: Glycosylated hemoglobin A1c; LDL-C: Low-density lipoprotein cholesterol; eGFR: Estimated glomerular filtration rate; Group C: Samples with non-diabetics; Group DM: Samples with diabetics; NS: Not significant.
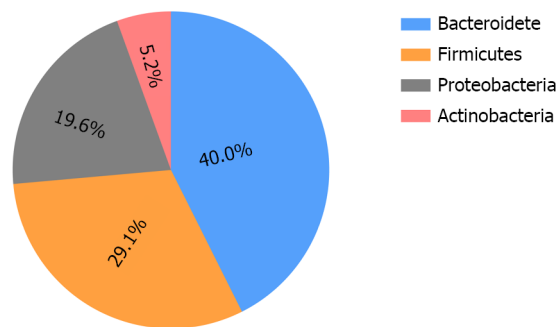
Figure 1 The pie shows average relative abundances of the most four dominated phyla. Relative abundances are presented as percentage.

The composition of fecal microbiota

At the phylum level, *Proteobacteria* was the most abundant in Group C followed by *Bacteroidetes* (Figure 4A). The abundance of *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* varied between Group DM and Group C (Figure 4A). The *Firmicutes* to *Bacteroidetes* ratio was slightly higher in Group DM compared with Group C, however, there was no significant difference (*r* = 0.86 *vs r* = 0.81, *P* = 0.53).

At the phylum level, the majority composition of microbiome in Group D and DR were *Bacteroidetes* and *Firmicutes* (Figure 4B). The relative mean abundance of phylum *Bacteroidetes* and *Firmicutes* as well as *Firmicutes* to *Bacteroidetes* ratio between Group D and Group DR were similar (*P* = 0.33, *P* = 0.37, *P* = 0.52, Figure 4B). The relative mean abundance of phylum *Bacteroidetes*, *Firmicutes* and *Firmicutes* to *Bacteroidetes* ratio showed a similarity between Group PDR and NPDR (*P* = 0.71, *P* = 0.33, *P* = 0.54, Figure 4C).

The linear discriminant analysis effect size revealed that *Fusob Cteriaceae*, *Fusobacteriales*, *Fusobacteriia*, *Fusobacteriaceae*, *Desulfovibrionales*, *Delta Proteobacteria*, *Burkholderiaceae* and *Beta Proteobacteriales* were dominant in Group DR (Figure 5A). Meanwhile, *Eubacteriaceae* and *Pseudomonadaceae* were dominant in PDR and Group NPDR respectively (Figure 5B).

Table 2 Basic characteristics compared between samples with diabetic retinopathy and samples without diabetic retinopathy, as well as patients with proliferative diabetic retinopathy *vs* patients without proliferative diabetic retinopathy

Characteristic	Group DR *vs* Group D			Group PDR *vs* Group NPDR		
	Group DR (*n* = 15)	Group D (*n* = 15)	*P* value	Group PDR (*n* = 8)	Group NPDR (*n* = 7)	*P* value
Age	55.87 ± 10.54	56.00 ± 8.90	NS	58.75 ± 11.85	52.57 ± 8.44	NS
Gender, male/female	9/6	10/5	NS	3/5	3/4	NS
Diabetes duration	14.27 ± 7.27	4.045 ± 4.67	NS	15.50 ± 4.99	12.87 ± 9.49	< 0.01
height	165.13 ± 9.26	166.27 ± 6.97	NS	165.75 ± 10.12	164.43 ± 8.90	NS
Weight	64.83 ± 9.11	64.67 ± 6.24	NS	65.19 ± 9.64	64.43 ± 9.22	NS
Body mass index	23.49 ± 1.99	23.45 ± 2.48	NS	23.07 ± 1.24	23.97 ± 2.62	NS
Waist circumference	83.47 ± 8.40	86.03 ± 7.17	NS	85.75 ± 9.77	80.86 ± 6.20	NS
Hip circumference	89.53 ± 8.83	93.30 ± 5.03	NS	92.88 ± 8.17	85.71 ± 8.50	NS
Waist hip ratio	0.93 ± 0.06	0.92 ± 0.05	NS	0.92 ± 0.07	0.95 ± 0.03	NS
Waist height ratio	0.51 ± 0.05	0.52 ± 0.05	NS	0.52 ± 0.07	0.49 ± 0.02	NS
Systolic blood pressure	131.93 ± 13.27	124.93 ± 9.74	NS	135.75 ± 13.79	127.57 ± 12.15	NS
Fasting glucose	9.24 ± 2.95	7.34 ± 2.35	NS	9.61 ± 1.83	8.81 ± 4.00	NS
HbA1c	10.01 ± 1.63	9.87 ± 2.31	NS	9.26 ± 1.62	10.87 ± 1.24	NS
Triglycerides	2.18 ± 2.50	1.44 ± 0.57	NS	2.42 ± 2.90	1.90 ± 2.14	NS
LDL-C	2.78 ± 0.90	3.22 ± 1.02	NS	2.51 ± 1.16	3.09 ± 0.34	NS
eGFR	88.08 ± 32.09	96.51 ± 22.72	NS	69.33 ± 33.84	109.51 ± 7.88	< 0.05
Urine albumin creatine ratio	527.22 ± 1055.30	30.90 ± 52.89	NS	508.40 ± 731.68	548.74 ± 1404.61	NS
Serum creatinine clearance value	97.59 ± 52.53	114.66 ± 41.75	NS	94.24 ± 60.68	101.41 ± 45.96	NS
Urea nitrogen	7.65 ± 5.66	5.87 ± 2.24	NS	9.60 ± 7.24	5.41 ± 1.58	NS
Fasting insulin	61.34 ± 24.06	70.91 ± 57.40	NS	73.31 ± 19.84	47.66 ± 21.96	< 0.05
HOMA-IR	3.82 ± 2.13	3.10 ± 2.00	NS	4.53 ± 1.62	23.00 ± 2.46	NS
HOMA-B	0.40 ± 0.25	0.80 ± 1.02	NS	0.36 ± 0.13	0.45 ± 0.36	NS
Platelet	217.60 ± 55.49	203.67 ± 63.63	NS	196.63 ± 43.45	241.57 ± 61.04	NS
Mean platelet volume	11.13 ± 0.85	10.99 ± 0.79	NS	11.40 ± 0.88	10.83 ± 0.76	NS
Platelet distribution width	13.79 ± 1.58	13.95 ± 1.55	NS	14.05 ± 1.55	13.49 ± 1.67	NS
Neutrophil to lymphocyte ratio	2.85 ± 0.99	2.10 ± 0.86	< 0.05	2.75 ± 0.90	2.97 ± 1.15	NS
T cell count	1165.87 ± 373.69	1518.00 ± 335.85	NS	1092.75 ± 504.02	1249.43 ± 119.08	NS
B cell count	249.67 ± 82.72	378.87 ± 124.49	< 0.01	198.00 ± 57.65	308.71 ± 66.81	< 0.01
CD4^{+} T cell count	733.87 ± 299.45	953.2 ± 226.40	< 0.05	684.13 ± 413.77	790.72 ± 49.25	NS
CD8^{+} T cell count	389.40 ± 116.23	490.47 ± 137.90	< 0.05	371.88 ± 145.83	409.43 ± 76.37	NS
CD4^{+} to CD8^{+} T cell ratio	1.91 ± 0.60	2.01 ± 0.42	NS	1.85 ± 0.79	1.98 ± 0.31	NS
NK cell count	220.20 ± 85.18	462.27 ± 250.54	< 0.01	240.00 ± 114.91	197.57 ± 20.12	NS
NK cell percent	12.66 ± 3.65	19.41 ± 9.48	< 0.05	14.05 ± 4.52	11.07 ± 1.33	NS

Data are presented as mean ± SD. HbA1c: Glycosylated hemoglobin A1c; LDL-C: Low-density lipoprotein cholesterol; eGFR: Estimated glomerular filtration rate; HOMA-IR: Homeostasis model assessment of insulin resistance; HOMA-B: Homeostasis model assessment of beta-cell function; NS: No significance; Group DR: Samples with diabetic retinopathy; Group D: Samples without diabetic retinopathy; Group PDR: Patients with proliferative diabetic retinopathy; Group NPDR: Patients without proliferative diabetic retinopathy.
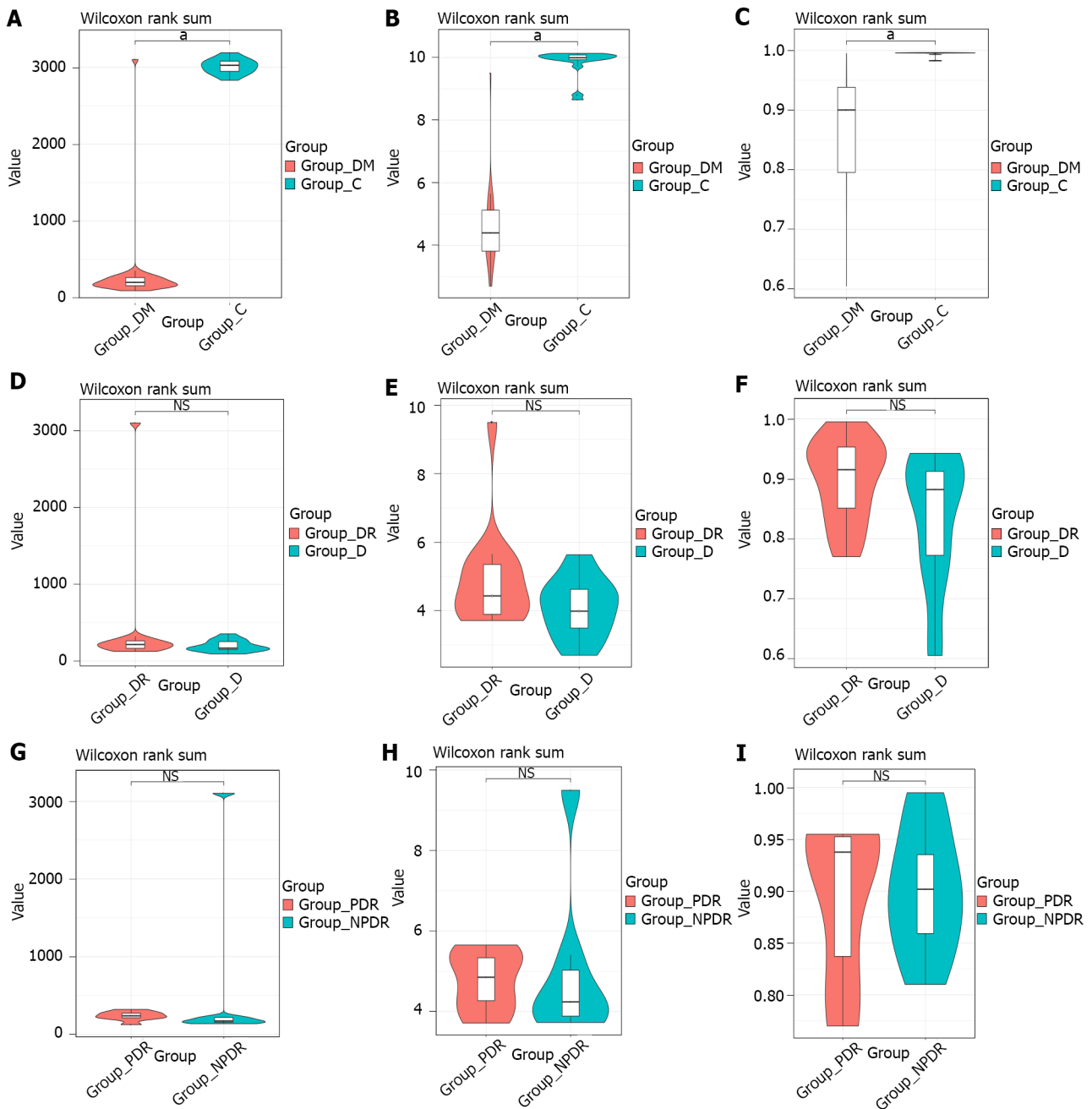
Figure 2 Alpha diversity analysis of microbiota in the six groups. A: The Chao1 index between Group DM and Group C; B: The Shannon index between Group DM and Group C; C: The simpson index between Group DM and Group C; D: The Chao1 index between Group DR and Group D; E: The Shannon index between Group DR and Group D; F: The simpson index between Group DR and Group D; G: The Chao1 index between Group PDR and Group NPDR; H: The Shannon index between Group PDR and Group NPDR; I: The simpson index between Group PDR and Group NPDR. [a]$P < 0.05$. NS: Not significant. Group C: Samples with non-diabetics; Group DM: Samples with diabetics; Group DR: Samples with diabetic retinopathy; Group D: Samples without diabetic retinopathy; Group PDR: Patients with proliferative diabetic retinopathy; Group NPDR: Patients without proliferative diabetic retinopathy.

At the phylum level, *Fusobacteria* was significant higher in Group DR than in Group D ($P < 0.05$, Figure 6A). In addition, at the family level, *Fusobacteriaceae*, *Burkholderiaceae*, *Desulfovibrionaceae* and *Pseudomonadaceae* were significantly increased in Group DR than in Group D ($P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.05$, Figure 6B). At the genus level, the abundance of *Senegalimassilia*, *S5-A14a* and *Lachnospiraceae_UCG-008* were significantly decreased in Group DR than in Group D ($P < 0.05$, $P < 0.05$, $P < 0.05$), whereas, *Fusobacterium*, *Pseudomonas*, *Lachnospiraceae_UCG-010* and *Adlercreutzia* were significantly increased ($P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.05$, Figure 6C). Further, *Eubacterium*, *Peptococcus*, *Desulfovibrio*, *Acetanaerobacterium*, *Negativibacillus* and *Family_XIII_UCG-001* were significantly increased in Group PDR compared with Group NPDR ($P < 0.01$, $P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.05$), whererea, *Pseudomonas*, *Alloprevotella*, *Tyzzerella* and *Tyzzerella-3* had a reduction ($P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.05$, Figure 6D).

A

Weighted PCoA

P < 0.01

Group
- Group_DM
- Group_C

PC2 11.84%

PC1 62.75%

B

Unweighted PCoA

P < 0.01

Group
- Group_DM
- Group_C

PC2 6.76%

PC1 53.46%

C

Weighted PCoA

P > 0.05

Group
- Group_DR
- Group_D

D

Unweighted PCoA

P > 0.05

Group
- Group_DR
- Group_D
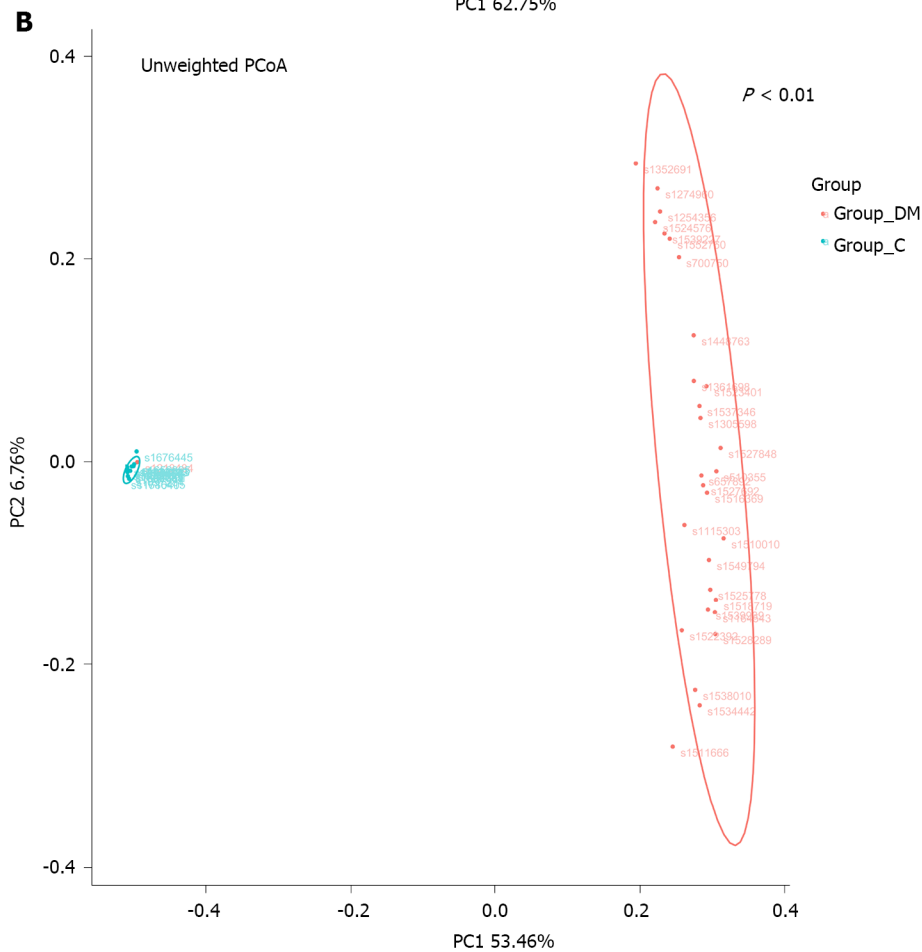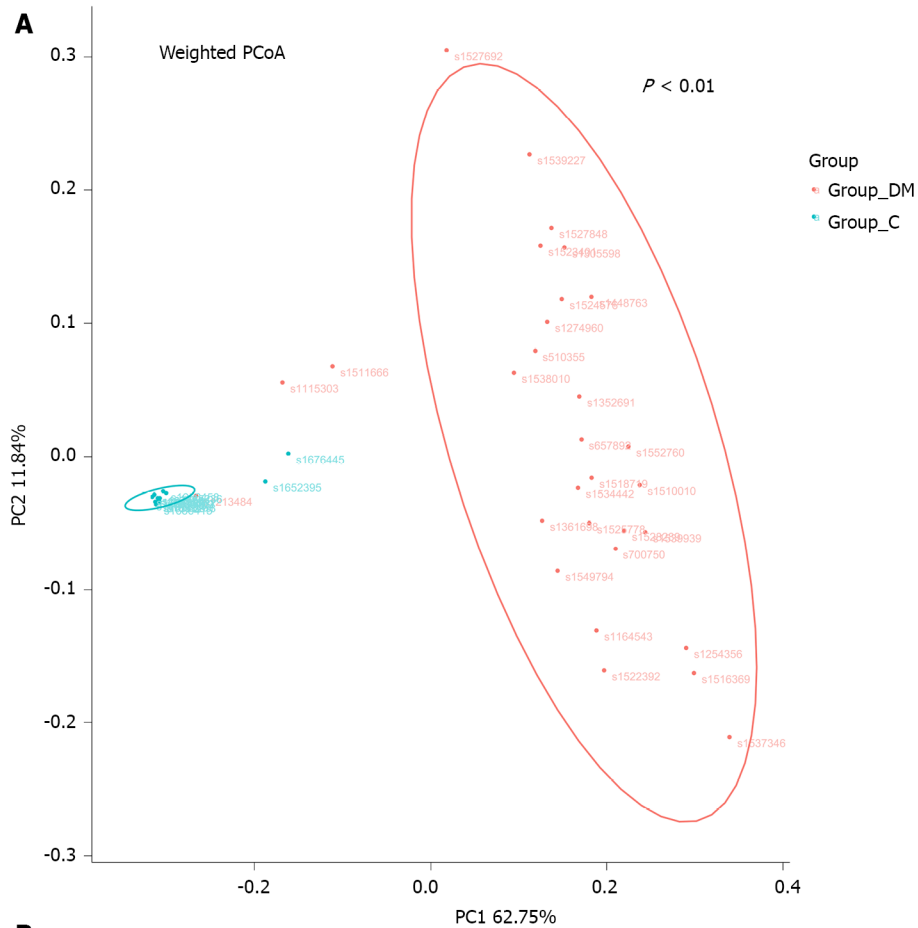
Figure 3 Beta diversity analysis of microbiota in the six groups. A and B: Weighted and unweighted PCoA between between Group DM and Group C; C

and D: Weighted and unweighted PCoA between between Group DR and Group D; E and F: Weighted and unweighted PCoA between between Group PDR and Group NPDR. Differences were assessed by Adonis. Group C: Samples with non-diabetics; Group DM: Samples with diabetics; Group DR: Samples with diabetic retinopathy; Group D: Samples without diabetic retinopathy; Group PDR: Patients with proliferative diabetic retinopathy; Group NPDR: Patients without proliferative diabetic retinopathy.
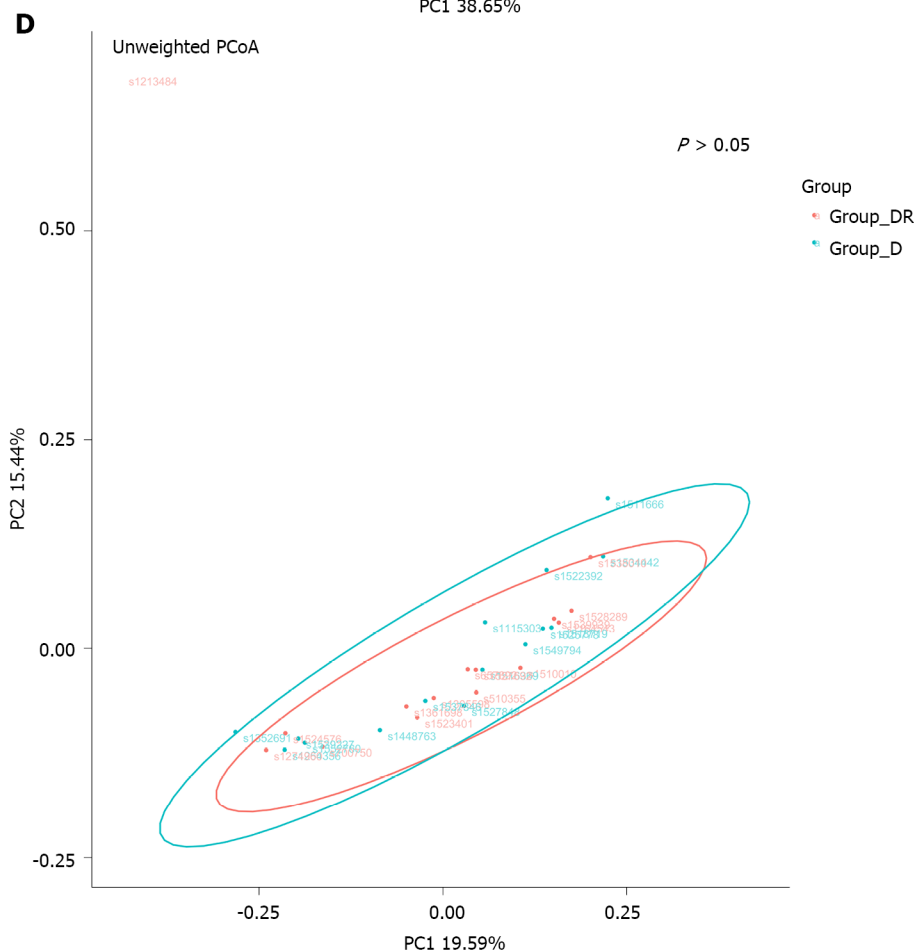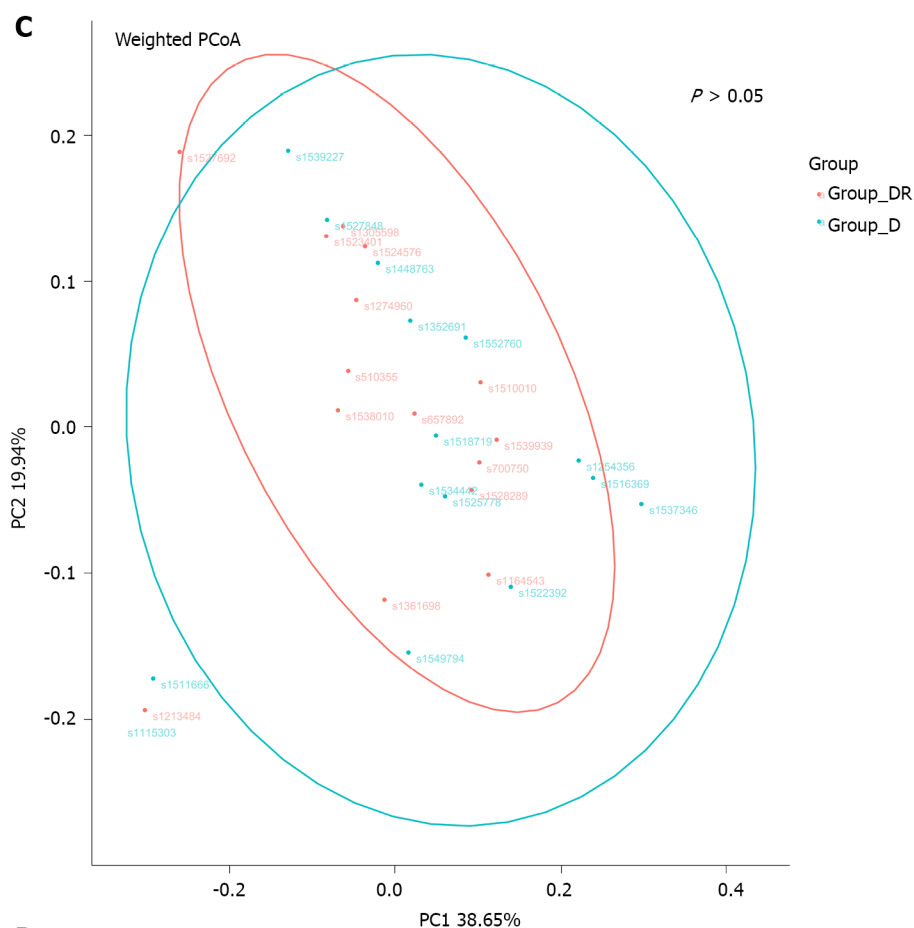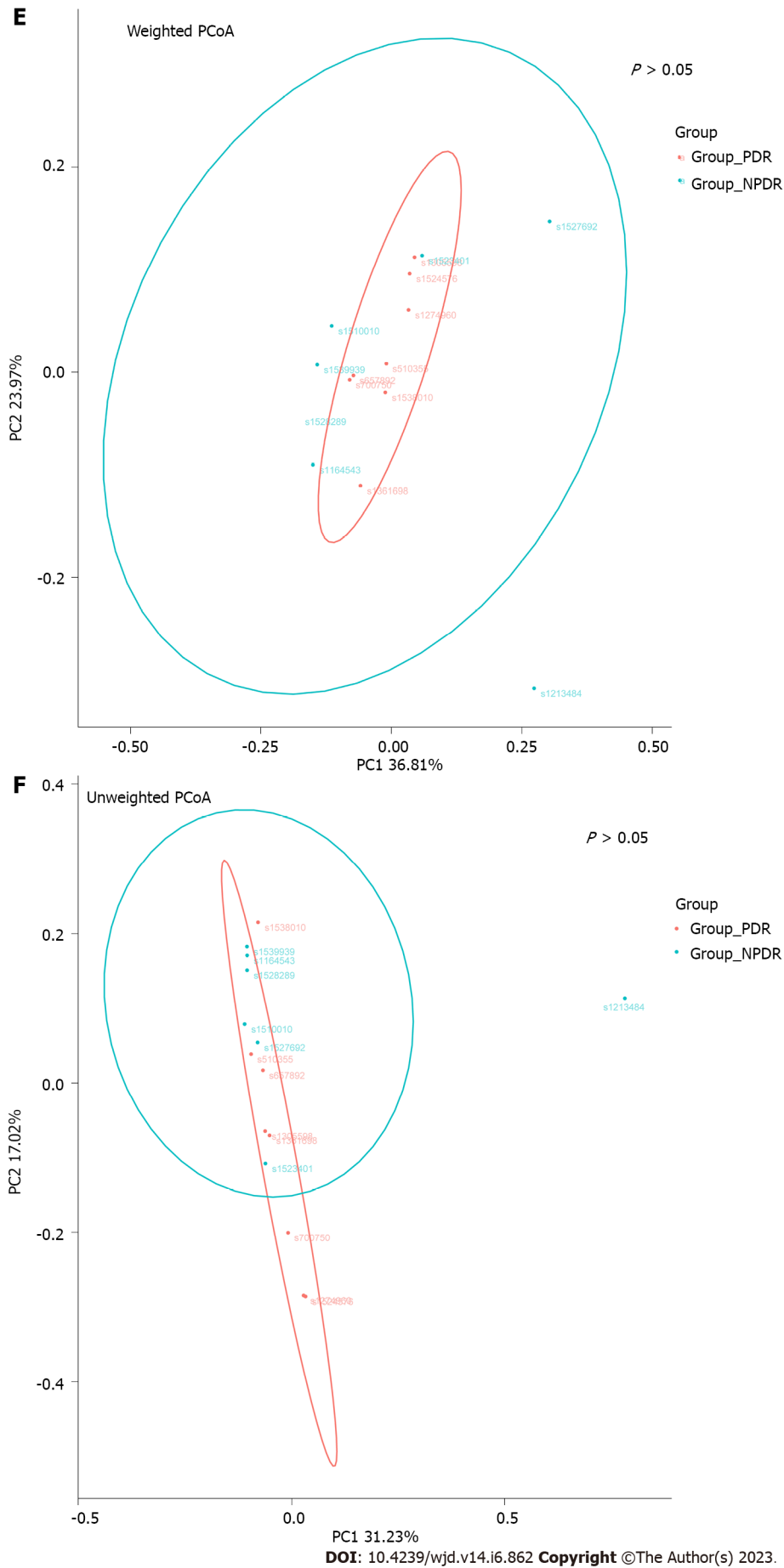
Spearman's correlations between the relative abundance of bacterial families, clinical indices and biochemical characteristics were performed between Group DR and Group D as well as Group PDR and NPDR. In Group D and Group DR, *Pseudomonas* had a negative correlation with NK cell count (r = -0.39, P < 0.05, Figure 7A). However, *Senegalimassilia* had a positive correlation with NK cell% (r = 0.42, P < 0.05, Figure 7A). Meanwhile, in Group PDR and Group NPDR, *Acetanaerobacterium* (r = 0.53, P < 0.05) and *Desulfovibrio* were positively correlated with fasting insulin (r = 0.61, P < 0.05, Figure 7B), when *Negativibacillus* was negatively correlated with B cell count (r = -0.67, P < 0.01) and eGFR (r = -0.66, P < 0.01, Figure 7C).

DISCUSSION

Disorder in intestinal microbiota composition has been implicated in occurrence and development of diabetes mellitus (DM)[36,37]. Intestinal microbiota dysbiosis induces oxidative stress, inflammation, insulin resistance and vascular permeability, which probably involves in progression of diabetic complication including DR[37,38]. However, the association between intestinal microbiota and DR remains unclear. Moubayed *et al*[20] found diabetic patients have higher relative abundance of Bacteroides than non-diabetic patients, however microbiota differences between patients with DR and without DR were not detected. A study presented microbiota biomarkers to help diagnosing DR, but not analyze the relation between them and clinical markers[22]. Considering existing mature examination to make a definite diagnosis of DR, microbiota biomarkers did not contribute much to the diagnosis. Besides, there was no evidence to prove that the relationship between biomarkers and DR was not accidental. The primary aim of the current study was to assess the gut flora differences of persons with DM and healthy controls, of diabetic patients with DR and without DR separately, applying 16S rRNA gene sequencing. In addition, the analysis of the correlation between the gut flora differences and clinical indexes was taken.

PCOA analysis revealed that the α diversity was decreased significantly in Group DM compared those in Group C, which was in line with previous study[22]. Lower bacterial richness was associated with several common metabolic markers including overall adiposity, insulin resistance and dyslipidaemia coexisted in T2DM[39]. Maintenance of gut nomobiosis played a protective role in glycolipid metabolism[40], on the contrary, gut dysbiosis characterised with reduced microbiota diversity induced expansion of pathogenic bacteria, gut inflammation and deterioration of diabetes[41,42]. Significant variations of gut microbiota between T2DM patients and nondiabetic controls revealed in PCoA was showed by a previous report[43], which was accordant with ours. In addition, in accordance with previous study, α diversity indexes did not differ significantly between Group DR and Group D[22,23]. The α and β diversity did not significantly change between Group DR and Group D as well as between Group NPDR and PDR in our study.

At phylum level, *Bacteroidota*, *Firmicutes*, *Proteobacteria* and *Actinobacteriota* occupied more than 80% of community abundance were regarded as the most dominant phyla in each group. A study found that the abundance of *Firmicutes* and *Bacteroidetes* increased in Group DM compared with Group C, with slightly higher *Firmicutes*/*Bacteroidetes* ratio in Group DM, consistent to our study[43]. Further, the investigation performed by Li *et al*[44] got the similar results in Han population. Of interests, we found that *Firmicutes* were more abundant in Group DR than Group D, while *Bacteroidetes* were less abundant in Group DR *via* Wilcoxon test. However, Huang *et al*[22] got an opposite result. Although the result in our study was lack of statistical difference, this distinction also got our attention. Metformin may influence *Firmicutes* abundance[45]. We guessed that metformin received by most diabetic patients without DR stated by Huang *et al*[22] might led to lower abundance of *Firmicutes* in Group DM without DR. Compared with Group D, there was a significant increase abundance of *Fusobacteria* in Group DR. However, the study performed by Sisinthy Shivaji was discordant with ours[23], which possibly due to the inconsistent effect of metformin on *Fusobacteria*[28].

Our results indicated that *Fusobacteriacee*, *Desulfovibrionaceae*, *Burkholderiaceae* and *Pseudomonadaceae* at the family level increased in Group DR compared with Group D. Further, *Eubacteriaceae* and *Pseudomonadaceae* were predominant in Group PDR and Group NPDR respectively. *Fusobacteria* and *Fusobacteriaceae* produced short-chain fatty acids including acetate and propionate and their abundance increased in non-alcoholic steatohepatitis (NASH) subjects compared to nonalcoholic fatty liver and healthy controls[46]. Elevated and propionate in faeces of human NASH subjects were relevant to the increase of Th17 in peripheral blood[46]. An study in animal model showed that blocking the IL-23-Th17-IL-17A pathway would help alleviating DR in mice[47]. Therefore, our results suggested that
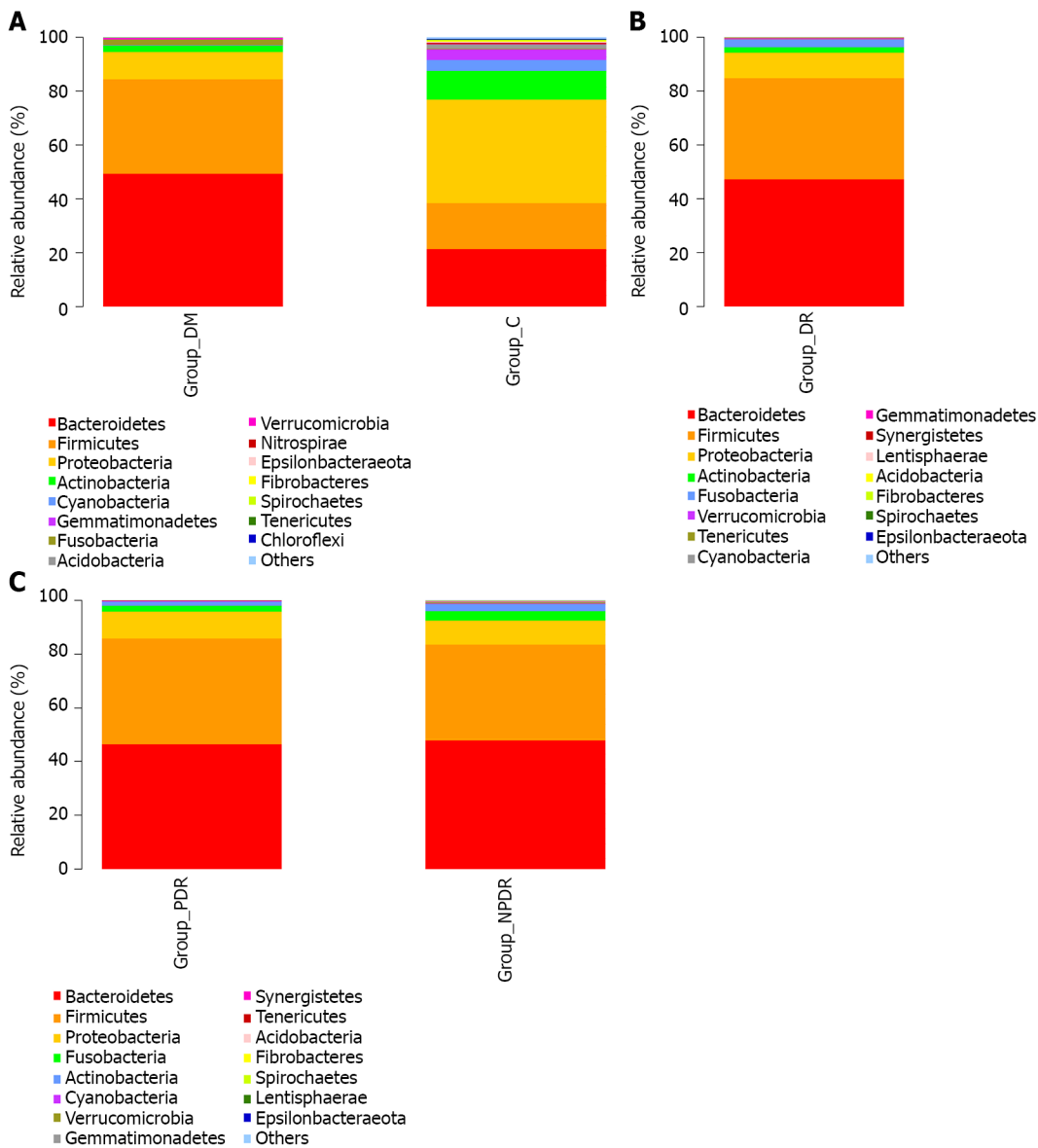
Figure 4 The composition at phylum level in the six groups. A: The composition at phylum level in Group DM and Group C. The abundance of *Firmicutes, Bacteroidetes, Proteobacteria* and *Actinobacteria* varied between the two groups; B: The composition at phylum level in Group DR and Group D. The relative mean abundance of phylum *Bacteroidetes* and *Firmicutes* between Group D and Group DR were similar (*P* = 0.33, *P* = 0.37); C: The composition at phylum level in Group PDR and Group NPDR. The relative mean abundance of phylum *Bacteroidetes*, *Firmicutes* and showed a similarity between Group PDR and NPDR (*P* = 0.71, *P* = 0.33). Group C: Samples with non-diabetics; Group DM: Samples with diabetics; Group DR: Samples with diabetic retinopathy; Group D: Samples without diabetic retinopathy; Group PDR: Patients with proliferative diabetic retinopathy; Group NPDR: Patients without proliferative diabetic retinopathy.

increased *Fusobacteria* and *Fusobacteriaceae* may contribute to producing acetate and propionate, increasing Th17 and causing DR. *Fusobacteriaceae* is a gram-negative bacterium producing endotoxin, LPS[48]. A study found that *Fusobacteriaceae* increased and induced LPS in pigs with NASH[48]. And, in hyperglycaemic mice, elevation of systemic LPS contributed to the occurrence of DR[49]. Thus, higher abundance of *Fusobacteriaceae* possibly produce LPS and cause DR in our study. *Eubacteriaceae* is one of the bacteria that can metabolize aromatic amino acids to produce p-Cresy lsulfate (a prototype protein-bound uremic toxin)[50]. P-Cresy lsulfate induced renal cell carcinoma to overexpress hypoxia-inducible factor (HIF)-1α[51]. In our study, eGFR was lower in Group PDR than NPDR. P-Cresy lsulfate which was one of metabolites of great uremic solutes produced by *Eubacteriaceae* may deteriorate DR by elevate the level of HIF-1α in retina in our study.

Compared to Group D, the genera *Fusobacterium*, *Pseudomonas*, *Adlercreutzia* and *Lachnospirace-ae_UCG-010* were increased, but *Senegalimassilia*, *Lachnospiraceae_UCG-008* and *S5-A14a* were decreased in Group DR. Huang *et al*[22] found that compared with patients with diabetic patients without DR , Group DR had decreased *Blautia* and *Lactobacillus* and less of them took metformin. Besides preventing the occurrence of DR, metformin also increase *Blautia* and *Lactobacillus*[27,52]. Accordingly, despite lack of statistical differences in the numbers of patients using metformin between the two groups stated by
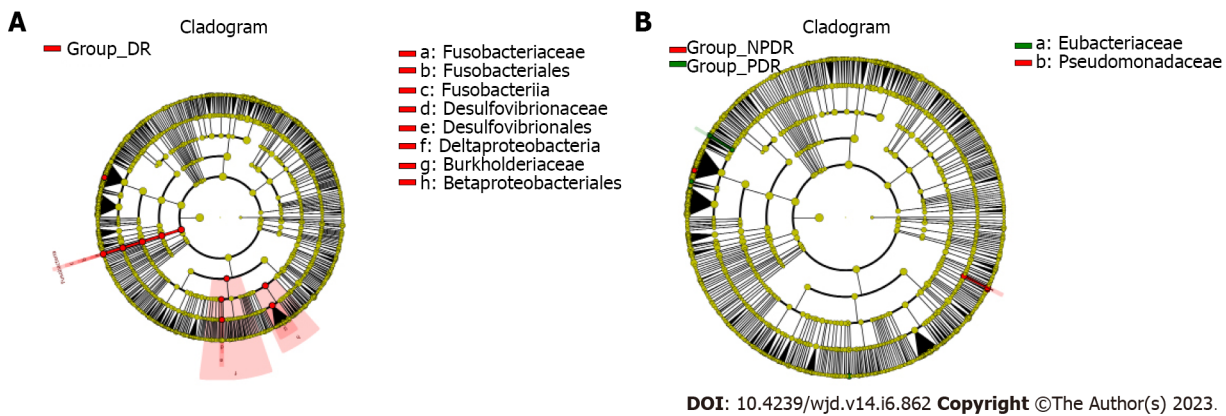
Figure 5 Results of linear discriminant analysis effect size in the six groups. Wilcoxon signed rank test was used for statistical analyzing. The threshold of linear discriminant analysis score was set to 2.0. A and B: Red nodes designated microorganism that only been detected in Group DR. The regions marked with yellow indicated no significant difference between Group DR and Group D as well as Group PDR and NPDR. Group DR: Samples with diabetic retinopathy; Group D: Samples without diabetic retinopathy; Group PDR: Patients with proliferative diabetic retinopathy; Group NPDR: Patients without proliferative diabetic retinopathy.

Huang *et al*[22], we speculated that increased *Blautia* and *Lactobacillus* and lower incidence of DR were relevant to metformin, and the causal relationship between *Blautia*, *Lactobacillus* and DR remained uncertain. Patients in Group DR had a larger proportion of *Fusobacterium*, suggesting a possible larger proportion of *Fusobacterium Nucleatum* (*Fn*) than in Group D. As a most frequent *Fusobacterium Specie*, *FN* secreted adhesins recognized vascular endothelial cell receptors and increased the vascular permeability contributing to the development of retinopathy possibly[53,54]. This may explain why diabetic patients with higher *Fusobacterium* are more likely to develop DR. *Pseudomonas aeruginosa* was one of *Pseudomonas species* which was a common pathogen in human body[55]. *Pseudomonas aeruginosa* could help secreting exotoxin to induce hyperpermeability and thrombosis of pulmonary vessels[56]. Besides, our study found that *Pseudomonas* was negatively correlated with absolute value of NK cells, consistent to other studies[57,58]. We presumed that *Pseudomonas* increased the permeability of retinal blood vessels and decreased NK cells, resulting in the occurrence of DR. Further experiments were needed to confirm the supposition. *Adlercreutzia* was positively correlated with leptin level which was positively correlated with the severity of DR[59,60]. Therefore, *Adlercreutzia* may promote the occurrence of DR by influencing leptin. *Senegalimassilia* had the genome that produced enterolactone, which was one of the two kinds of lignans in mammals and negatively correlated with white blood cells and C-reactive protein[50,61,62]. Increased *Senegalimassilia* would inhibit inflammatory response by producing enterolactone, and prevent DR consequently. The relationship between *Lachnospiraceae_UCG-010*, *Lachnospiraceae_UCG-008*, *S5-A14a* and diabetic complications had not been reported.

Eubacterium, *Peptococcus*, *Desulfovibrio*, *Acetanaerobacterium*, *Negativibacillus* and *Family_XIII_UCG-001* were higher in Group PDR compared to Group NPDR, while *Pseudomonas*, *Alloprevotella* and *Tyzzerella* were lower. *Eubacterium* is known as a butyrate producer[63]. Sodium butyrate in low concentration can promote angiogenesis whereas high concentration sodium butyrate has anti-angiogenic effect[64,65]. However, whether *Eubacterium* in our study promotes DR by generating low concentration sodium butyrate needs to be further clarified. Diabetes had a close relationship with cognitive impairment[66-68]. Diabetics with DR were more likely to suffer cognitive impairment (CI) and patients with higher severity of DR were more likely to have higher incidence of CI[69]. In addition, retinal vessel and cerebral small vessel had similar embryological origin, size and structure, suggesting that DR and CI may have similar pathophysiological basis[69]. A study showed patients with T2DM who had CI had higher level of *Peptococcus* and our study showed patients in Group PDR had a higher level of *Peptococcus* than Group NPDR[70]. *Peptococcus* may inspire both CI and DR progression in diabetic patients. *Desulfovibrio* desulfuricans was one of three species isolated from human faeces and could induce endothelial cell to produce vascular cell adhesion molecule-1 (VCAM-1) relating to the severity of DR[71,72]. Elevated level of *Desulfovibrio desulfuricans* may involve in the progression of PDR. The conclusion needs to be further explored due to the lack of analyzing species levels in genera *Desulfovibrio*. *Desulfovibrio* and *Acetanaerobacterium* were positively correlated with fasting insulin level and their abundance were higher in Group PDR than Group NPDR in our study. Insulin could induce HIF and neovascularization by PI3K and MAPK pathway[73]. Hyperinsulinemia may be the mechanism of *Desulfovibrio* and *Acetanaerobacterium* promoting PDR. Our study showed that *Negativibacillus* was negatively correlated with B lymphocyte. Considering immune cells including B lymphocyte inhibited the formation of pulmonary neovascularization by ischemi, *Negativibacillus* may promote retinal neovascularization by decreasing B lymphocyte[74]. As stated earlier, *Pseudomonas* aeruginosa increased the incidence of DR by promoting increased vascular permeability. However, *Pseudomonas* aeruginosa
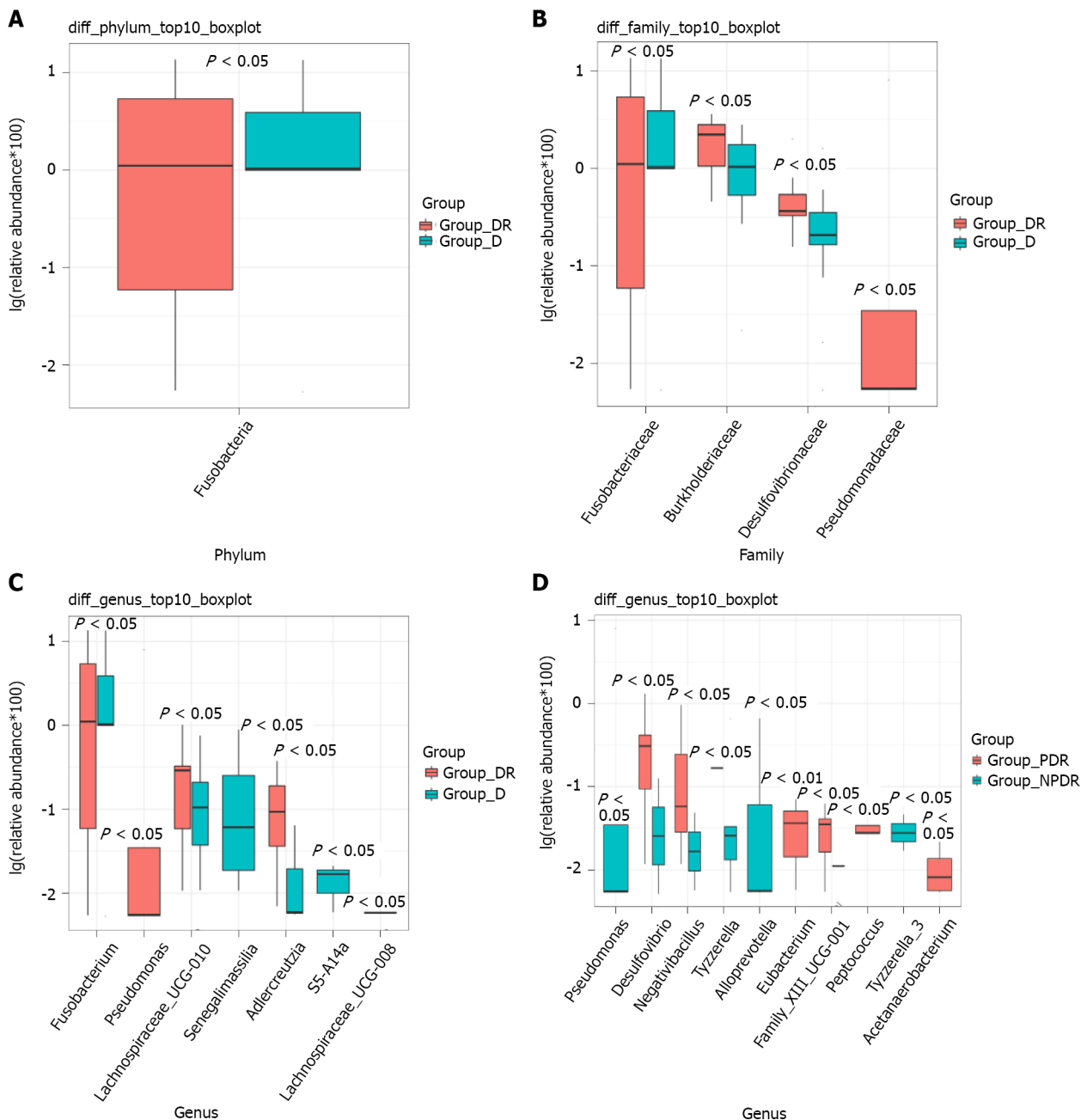
DOI: 10.4239/wjd.v14.i6.862 **Copyright** ©The Author(s) 2023.

Figure 6 Relative abundance of microbiota displayed by Box and whiskers plots. A-C: Box and whiskers plots display relative abundance of microbiota in different level between Group DR and Group D; D: Box and whiskers plots display relative abundance of genera between Group PDR and NPDR. Group DR: Samples with diabetic retinopathy; Group D: Samples without diabetic retinopathy; Group PDR: Patients with proliferative diabetic retinopathy; Group NPDR: Patients without proliferative diabetic retinopathy.

decreased in Group PDR than Group NPDR. *Pseudomonas* aeruginosa inhibited HIF, a key molecule of developing PDR[75]. Reason for the phenomenon that *Pseudomon*as aeruginosa was related higher incidence of DR but lower incidence of PDR needed further exploration. Butyric acid exhibited anti-angiogenic effect by inhibit expression of *VEGF/KDR* gene, and the higher abundance of *Alloprevotella* in the Group NPDR may suppress angiogenesis *via* butyric acids, thus delay the onset of PDR[76]. *Tyzzerella* produced much propionate which was capable of reducing the expression of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) induced by cytokine[77,78]. The levels of VCAM-1 and ICAM-1 in serum and eyes of patients in Group PDR were elevated compared with Group NPDR[79]. Hence, *Tyzzerella* may have slowed the progression of DR by reducing VCAM-1 and ICAM-1. The effect of *Family XIII UCG-001* on DR was still unknown.
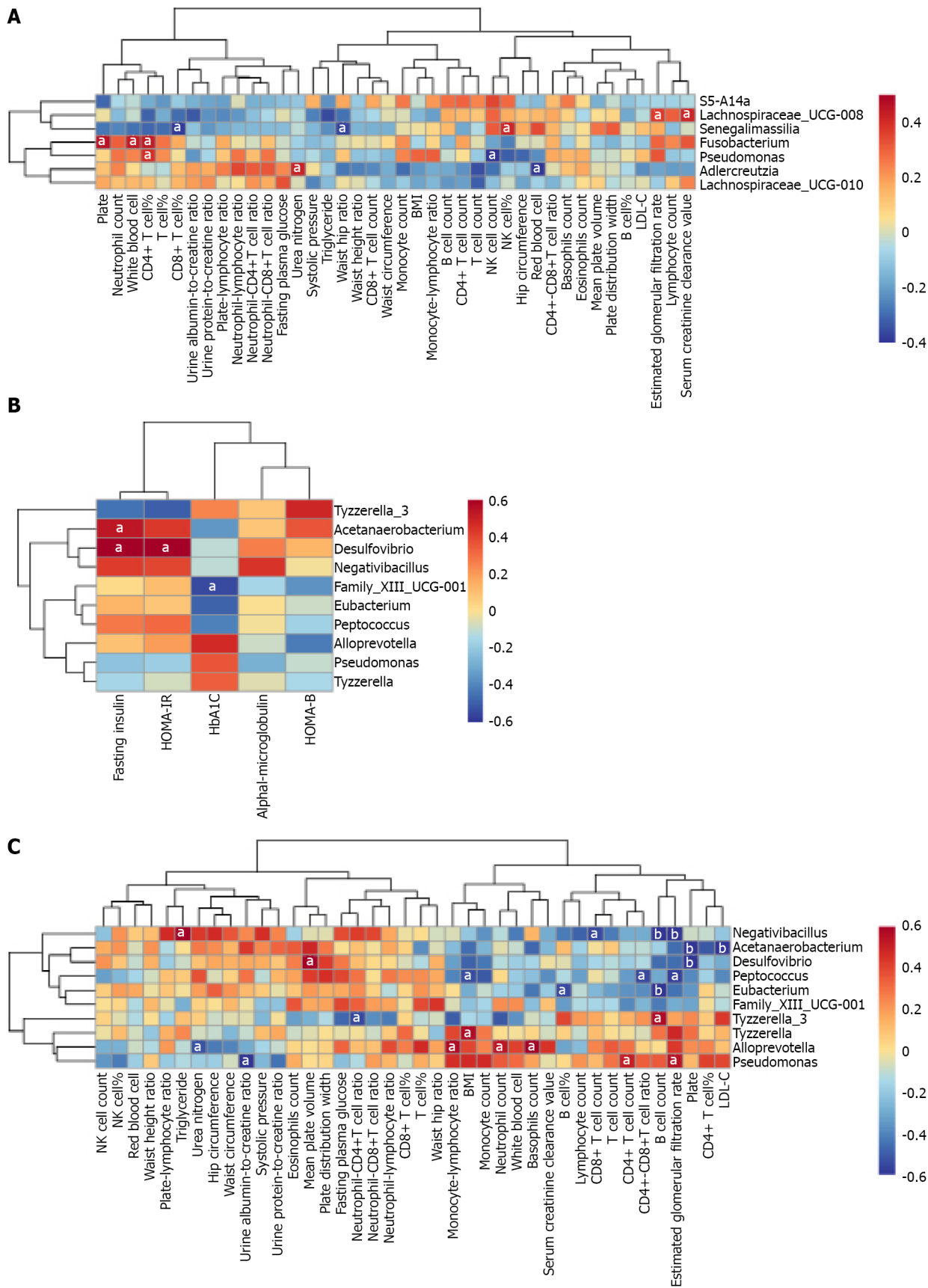
Figure 7 Correlation heatmap between gut microbiota and clinical indices. A: Correlation heatmap between gut microbiota and clinical indices in Group DM (Group DR *vs* Group D); B and C: Correlation heatmap between gut microbiota and clinical indices in Group DR (Group PDR *vs* Group NPDR). Different colors represent correlation level. Different colors represent correlation level (blue represents for negative correlation, red represents for positive correlation). [a]*P* < 0.05; [b]*P* < 0.01. Group C: Samples with non-diabetics; Group DM: Samples with diabetics; Group DR: Samples with diabetic retinopathy; Group D: Samples without

diabetic retinopathy; Group PDR: Patients with proliferative diabetic retinopathy; Group NPDR: Patients without proliferative diabetic retinopathy.

CONCLUSION

Our study explored the differences of intestinal microbiota between group DR and group D, as well as group PDR and group NPDR in the Chinese population of the southeast coastal region, rid of the interference of metformin. At the family level and genus level, much different microbiota was found between group DR and group D, and they may promote the occurrence of DR by affecting immune cells mediated by short-chain fatty acids, pro-inflammation response or anti-inflammation, inducing HIF and influencing permeability of blood vessels in the fundus. On the genus level, we found that besides *Pseudomonas*, the variation of microbiota composition between group PDR and group NPDR was completely different from that between group DR and group D. Some differential bacteria between group PDR and group NPDR may affect the level of butyrate or butyric acid, participate in the production of VCAM-1, decrease the level of HIF, affect the brain-eye barrier, promote insulin secretion and reduce B lymphocytes to promote or postpone the progress of DR. Accordingly, we speculated that the disorder of intestinal microbiota may be involved in the occurrence and development of DR, providing a possible novel therapeutic target for DR. However, our study lacked the detection at species level, as well as the measurement of microbial metabolites and related clinical indicators. The causal relationship between intestinal microbiota and the occurrence and development of DR remained unclear. Consider the limitation mentioned above, further investigation was required.

ARTICLE HIGHLIGHTS

Research background

For the therapy of diabetic retinopathy (DR), current approaches showed their own limitations. Modulation of gut flora was capable of preventing DR, which was revealed by animal experiment.

Research motivation

To provide clues for novel ways to prevention and treatment methods of DR.

Research objectives

This study aims to explore the relationship between intestinal microbiota and DR among patients in the southeast coast of China.

Research methods

By 16S rRNA sequencing, fecal samples of non-diabetics (Group C, n = 15) and diabetics (Group DM, n = 30) were analyzed. Spearman correlation analyses were performed to explore the associations between intestinal microbiota and clinical indicators.

Research results

The alpha and beta diversity did not differ significantly between Group DR and Group D as well as Group PDR and Group NPDR. At the genera level, Pseudomonas, Fusobacterium and Adlercreutzia were increased in Group DR than Group D while Senegalimassilia was decreased (P < 0.05, respectively). At the family level, Pseudomonadaceae, Desulfovibrionaceae and Fusobacteriaceae were significantly increased in Group DR than in Group D (P < 0.05, respectively). Pseudomonas was negatively correlated with NK cell count (r = -0.39, P = 0.03). In addition, the abundance of Pseudomonas, Alloprevotella and Tyzzerella (P < 0.05, respectively) were lower in Group PDR compared to Group NPDR, while genera Eubacterium (P < 0.01), Peptococcus, Desulfovibrio, Acetanaerobacterium and Negativibacillus (P < 0.05, respectively) were higher. Desulfovibrio and Acetanaerobacterium were positively associated with fasting insulin (r = 0.53 and 0.61, respectively, P < 0.05), when Negativibacillus was negatively associated with B cell count (r = −0.67, P < 0.01).

Research conclusions

Our research revealed that dysbiosis of gut flora was correlated with DR and its progression among diabetics in the southeast coast of China, probably *via* several mechanisms including producing influencing permeability of blood vessels, short-chain fatty acids, affecting levels of vascular cell adhesion molecule-1, hypoxia-inducible factor-1, B cell and insulin. Manipulating gut microbiota might be a novel way for prevention of DR, particularly PDR in population above.

Research perspectives

This research perspectives are as fellow: (1) Current treatments for DR did not acquire satisfied effect; (2) Animal experiment revealed that reconstruction of gut microbiota could prevent DR; and (3) Does alteration of gut microbiota has connection with DR in human?

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Retrospective Study

Application of urinary N-acetyl-β-D-glucosaminidase combined with serum retinol-binding protein in early detection of diabetic nephropathy

Zhi-Hao Lin, Sheng-Feng Dai, Jian-Nan Zhao, Yue Jiang

Zhi-Hao Lin, Sheng-Feng Dai, Department of Endocrinology, Wenzhou Hospital of Traditional Chinese Medicine Affiliated to Zhejiang University of Traditional Chinese Medicine, Wenzhou 325000, Zhejiang Province, China

Jian-Nan Zhao, Department of Endocrinology, Yongjia Hospital of Traditional Chinese Medicine, Wenzhou 325000, Zhejiang Province, China

Yue Jiang, Department of Acupuncture, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

Corresponding author: Yue Jiang, MD, Occupational Physician, Department of Acupuncture, The First Affiliated Hospital of Wenzhou Medical University, Shangcai Village, Nanbaixiang Street, Ouhai District, Wenzhou 325000, Zhejiang Province, China. jyygl9397@126.com

Abstract

BACKGROUND
Diabetic nephropathy (DN) is a microangiopathy of type 2 diabetes mellitus (T2DM), which can damage the kidney through various ways and mechanisms due to the nature of the disease, involving the renal interstitium and glomeruli. However, in the early stage of the disease, patients only showed kidney volume increase and glomerular hyperthyroidism, and typical symptoms that are difficult to arouse individual attention were noticed.

AIM
To observe the expression of serum retinol-binding protein (RBP) and urinary N-acetyl-β-D-glucosaminidase (NAG) in patients with DN, and to analyze their value in disease prediction, so as to provide new targets for early diagnosis and treatment of DN.

METHODS
The baseline data of 50 T2DM patients treated in our hospital between January 2021 and December 2022 were retrospectively reviewed and included in group A. The baseline data of 50 patients with type 2 DN admitted to our hospital during the same period were collected and included in group B. The baseline data and serum RBP and urine NAG expression were compared between the two groups to analyze their value in the early prediction of DN.

RESULTS

There was no significant difference in age, gender, duration of diabetes, combined hyperlipidemia and combined hypertension between the two groups ($P > 0.05$); the expression of urinary NAG and serum RBP in group B was higher than that in group A, and the difference was statistically significant ($P < 0.05$); a multiple logistic regression model was established, and the results showed that urinary NAG and serum RBP were related to the presence or absence of injury in diabetic patients, and overexpression of urinary NAG and serum RBP may be risk factors for renal injury in T2DM patients (OR > 1, $P < 0.05$); receiver operating curve curve was plotted, and the results showed that the area under the curve of urinary NAG and serum RBP expression alone and in combination for predicting DN was > 0.80, and the predictive value was satisfactory; bivariate Spearman linear correlation analysis showed that there was a positive correlation between urinary NAG and serum RBP expression in patients with DN ($r = 0.566$, $P = 0.000$).

CONCLUSION

The increased expression of urinary NAG and serum RBP may be the risk factors leading to the progression of T2DM to DN. The possibility of DN can be considered in patients with urinary NAG and serum RBP overexpression by examining the expression of urinary NAG and serum RBP in patients with T2DM in clinical practice.

Core Tip: Retinol-binding protein (RBP) can combine with thyroid transporters to form polymer complexes, and activated RBP can free in plasma, pass through glomerular filtration, and be absorbed and decomposed by renal tubules. N-acetyl-β-D-glucosaminidase is a high molecular glycoprotein acidic hydrolase, which is an intracellular lysosomal enzyme mainly present in body fluids, organ tissues and blood cells of the body, and has a high expression especially in the proximal renal tubules, thus being clinically used as an important indicator for the evaluation of renal tubular function.

INTRODUCTION

Diabetic nephropathy (DN) is a microangiopathy of type 2 diabetes mellitus (T2DM), which is caused by many factors such as hemodynamics, glucose metabolism mechanism, oxidative stress, resulting in relative or absolute lack of insulin in the body. Patients mainly have persistent elevated blood glucose, nutritional metabolism disorders. DN can damage the kidney through various ways and mechanisms due to the nature of the disease, involving the renal interstitium, glomeruli, resulting in pathological changes in the kidney, such as glomerulosclerosis, but the initial manifestations of patients are only increased kidney volume, glomerular hyperfunction, not easy to appear the typical symptoms that attract individual attention, only in patients with edema, proteinuria caused detection, but at this time the disease has progressed to the irreversible stage, the best time of treatment is missed, the prognosis of patients is mostly unsatisfactory[1-3]. Therefore, it is particularly important to find new clinical biochemical factors or examination methods to help the early detection of patients with clinical DN to guide the development of early intervention means and improve the prognosis of patients. It has been reported that tubular injury is earlier than glomerular injury in patients with DN, suggesting that tubular injury-related indicators are more significant for guiding the early detection of DN[4-6]. Urine N-acetyl-β-D-glucosaminidase (NAG) is a hot indicator in the diagnosis and treatment of kidney-related diseases at present, and it has more research value in reflecting kidney injury, especially tubular injury [7-9]. Retinol-binding protein (RBP) is a transporter of retinol in blood and has significant value in the assessment of proximal tubular reabsorption function and glomerular filtration performance[10-12]. Based on the biological mechanism of the above two indicators in the body, consider whether they can be used as early diseases in patients with DN. In view of this, this study will focus on observing the expression of serum RBP and urinary NAG in patients with DN, and analyze the value of the two

indicators in disease prediction, providing a new target for early diagnosis and treatment of patients with DN.

MATERIALS AND METHODS

General data

Retrospective analysis was performed to collect the baseline data of 50 patients with T2DM admitted to our hospital between January 2021 and December 2022, and were included in group A. Within the group, there were 30 males and 20 females; the mean age was (43.12 ± 5.02) years. Baseline data were collected from 50 patients with type 2 DN admitted to our hospital during the same period and included in Group B, Within the group, there were 28 males and 22 females; the mean age was (43.25 ± 5.12) years.

Inclusion and exclusion criteria

Inclusion criteria: (1) The diagnosis of T2DM refers to the contents in the [Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes (2013 Edition)][13], which is clinically confirmed by oral glucose tolerance test; (2) Patients with DN refer to the contents in [the Expert Consensus on the Clinical Diagnosis of Diabetic Kidney Disease in Chinese Adults][14]; (3) No other related diseases of the kidney, such as acute and chronic nephritis; and (4) The relevant treatments involved in this study are properly preserved.

Exclusion criteria: (1) Combined endocrine diseases, such as thyroid disease; (2) Combined tumor, tuberculosis and other cachexia; (3) Patients with kidney damage caused by other reasons, such as long-term drug history; and (4) Patients with low compliance caused by combined psychological or mental disorders, who cannot successfully cooperate with the study.

Baseline data collection method

According to the study objectives and methods, a statistical table of general data was designed, which mainly included duration of diabetes, gender, age, combined hyperlipidemia and combined hypertension. Participants in this study all came from the same region.

Test methods for laboratory indicators

5 mL of fasting venous blood was collected at a rate of 3500 r/min with a radius of 15 cm, and the supernatant was obtained after centrifugation for 5 min. Serum RBP was measured by immunoturbidimetry (Beckman AU5800 automatic biochemical analyzer). Patients were asked to randomly obtain 5 mL of morning midstream urine, centrifuged at 1500 r/min, and the supernatant was obtained after 10 min of centrifugation to detect urinary NAG by colorimetry (kit produced by Beijing Jiuqiang Company). All the above operations were carried out in strict accordance with the instructions of relevant instruments, reagents.

Statistical analysis

Data processing was performed using SPSS 24.0 software, and all measurement data were tested for normality by Shapiro-Wilk test, and data that conformed to the normal distribution were expressed as mean ± SD, and comparisons between groups were performed using the independent samples t-test; "%" was used for enumeration data and expressed as χ^2 Test, correlation analysis was performed using bivariate Spearman line, and logistic regression analysis was used to test the relationship between urinary NAG and serum RBP expression and patients with DN; receiver operating curve (ROC) was plotted to test the value of urinary NAG and serum RBP in predicting DN, evaluated by area under the curve (AUC), AUC ≤ 0.50: No predictive value; 0.50 < AUC ≤ 0.70: Low predictive value; 0.70 < AUC ≤ 0.90: Moderate predictive value; AUC > 0.90: High predictive value; $P < 0.05$ was considered statistically significant.

RESULTS

Comparison of data between the two groups

There was no significant difference in age, gender, duration of diabetes, combined hyperlipidemia and combined hypertension between the two groups ($P > 0.05$); urinary NAG and serum RBP expression in group B were higher than those in group A, and the difference was statistically significant ($P < 0.05$, Table 1).

Table 1 Comparison of data between two groups

Indicators	Group A (*n* = 50)	Group B (*n* = 50)	Statistical value	*P* value
Gender, *n* (%)			$\chi^2 = 0.164$	0.685
Male	30 (60.00)	28 (56.00)		
Female	20 (40.00)	22 (44.00)		
Age (mean ± SD, yr)	43.12 ± 5.02	43.25 ± 5.12	*t* = 0.128	0.898
Duration of diabetes (mean ± SD, yr)	2.1 5 ± 0.52	2.23 ± 0.55	*t* = 0.747	0.457
Combined hyperlipidemia, *n* (%)			$\chi^2 = 0.164$	0.685
Yes	20 (40.00)	22 (44.00)		
No	30 (60.00)	28 (56.00)		
Combined hypertension, *n* (%)			$\chi^2 = 0.170$	0.680
Yes	18 (36.00)	20 (40.00)		
No	32 (64.00)	30 (60.00)		
Urine NAG (mean ± SD, U/L)	14.05 ± 2.20	19.45 ± 3.68	*t* = 8.906	< 0.001
Serum RBP (mean ± SD, mg/L)	43.56 ± 5.50	84.98 ± 15.70	*t* = 17.606	< 0.001

NAG: N-acetyl-β-D-glucosaminidase; RBP: Retinol-binding protein.

Logistic regression analysis of relationship between urinary NAG, serum RBP and DN

Serum RBP and urine NAG of the included subjects were used as covariates, and the conditions of the included subjects were used as dependent variables (1 = DN, 0 = T2DM). After binary regression analysis, all the data in 2.1 were included to establish a multiple logistic regression model. The results showed that urine NAG and serum RBP were related to the presence or absence of injury in diabetic patients, and overexpression of urine NAG and serum RBP may be risk factors for renal injury in T2DM patients (OR > 1, *P* < 0.05, Table 2).

Value analysis of urinary NAG and serum RBP expression in predicting patients with DN

Urinary NAG and serum RBP expression of the included subjects were used as test variables, and the conditions of the included subjects were used as state variables (1 = DN, 0 = T2DM) to draw ROC curves (Figure 1), and the results showed that the AUC of urinary NAG and serum RBP expression alone and in combination in predicting DN were > 0.80, with satisfactory predictive value (Table 3).

Correlation analysis between urinary NAG and serum RBP expression in patients with DN

Bivariate Spearman linear correlation analysis showed a positive correlation between urinary NAG and serum RBP expression in patients with DN (*r* = 0.566, *P* = 0.000).

DISCUSSION

DN is a common complication in patients with T2DM. Urinary albumin, creatinine, blood urea nitrogen and other indicators have been used to assess whether diabetic patients have kidney damage. However, since kidney has self-compensation effect, indicators do not show significant changes in early stage renal impairment, the sensitivity of these indicators is low, and the above indicators can detect abnormalities only when the collective kidney has been damaged. However, irreversible damage has occurred in the body kidney at this stage, resulting in difficulty in the early detection of DN[15-17]. In view of this, many clinical reports have pointed out that inflammatory response, polyol metabolic pathway, abnormal changes in renal hemodynamics, oxidative stress and other mechanisms are related to the occurrence and disease progression of patients with DN, in the process of occurrence and progression of DN, there are renal tubular reabsorption dysfunction, glomerular filtration changes, and abnormal changes of multiple molecules in blood and urine. Thus, whether other indicators in serum or urine can be used as early detection of patients with clinical DN[18-20].

RBP is a carrier protein synthesized and secreted by stem cells, which is mainly synthesized by carbohydrates and a polypeptide chain, and has a very short half-life, which is necessary to help vitamin A transport on hepatocytes to epithelial cells. In many plasma, RBP can bind to thyroid transporter to form a polymer complex. Activated RBP can be free in plasma and filtered by glomeruli, where most of

Table 2 Logistic regression analysis of the relationship between urinary N-acetyl-β-D-glucosaminidase, serum retinol-binding protein and diabetic nephropathy

Variable	B	SE	Wals	P value	OR	95%CI Upper limit	95%CI Lower limit
Constant	-9.366	1.808	26.839	0.000	0.000	-	-
Urine NAG (U/L)	0.568	0.111	26.338	0.000	1.765	1.421	2.192
Serum RBP (mg/L)	0.346	0.109	9.996	0.002	1.413	1.141	1.751

NAG: N-acetyl-β-D-glucosaminidase; RBP: Retinol-binding protein.

Table 3 Efficacy analysis of urinary N-acetyl-β-D-glucosaminidase and serum retinol-binding protein expression for predicting diabetic nephropathy

Indicators	AUC	95%CI of AUC	SE	P value	Cut-off value	Specificity	Sensitivity	Youden index
Urine NAG	0.867	0.796-0.939	0.036	0.000	11.855 (U/L)	0.980	0.860	0.840
Serum RBP	0.951	0.902-1.000	0.025	0.000	39.620 (mg/L)	0.980	0.780	0.640
Combined diagnosis	0.974	0.936-1.000	0.020	0.000	-	0.980	0.940	

NAG: N-acetyl-β-D-glucosaminidase; RBP: Retinol-binding protein; AUC: Area under the curve.

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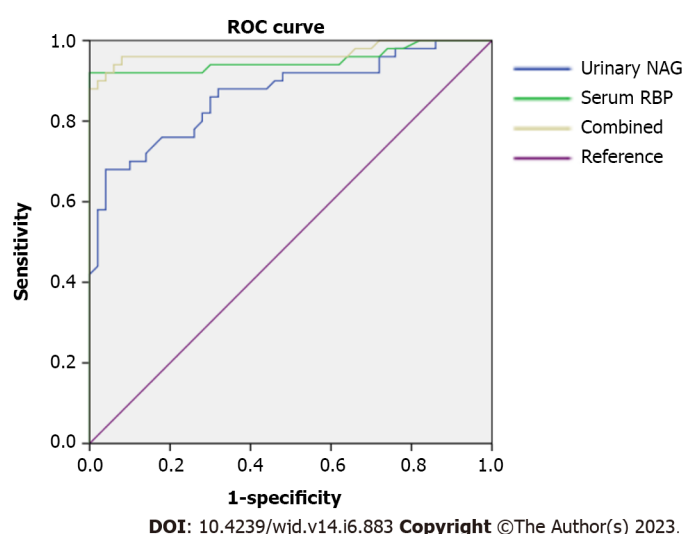
Figure 1 Receiver operating curve of urinary N-acetyl-β-D-glucosaminidase and serum retinol-binding protein expression for predicting diabetic nephropathy. ROC: Receiver operating curve; NAG: N-acetyl-β-D-glucosaminidase; RBP: Retinol-binding protein.

RBP is absorbed and decomposed by the proximal renal tubules for normal use by tissues, and only a few is excreted in the urine, so the level detected in serum or urine is extremely low under healthy conditions[21-23]. The changes of RBP content suggest the pathological changes of renal tubules and glomeruli. Under the action of induction factors, RBP can stimulate oxidative stress in the body and increase the damage of oxygen free radicals to the vascular endothelium[24-26].

NAG is a large lysosomal molecule present in tubular epithelial cells and does not efficiently pass through the glomerular filtration membrane[27-29]. NAG is a high molecular glycoprotein acid hydrolase, an intracellular lysosomal enzyme mainly present in body fluids, organ tissues and blood cells, especially highly expressed in the proximal renal convoluted tubules, and is clinically used as an important indicator for tubular function assessment[30-32]. In a healthy state, cause NAG has a large molecular weight and cannot normally pass through glomerular filtration, the renal tubules in the early stage of DN can still absorb the excessive proteinuria of glomerular filtration. Urine albumin in this stage is normal, but the expression of NAG increases, which may be due to the strengthening of reabsorption by the renal proximal convoluted tubule, the high protein content in the renal proximal

convoluted tubule stimulating the reabsorption system, activation of mitochondrial lysosomal enzyme, and increased lysosomal enzyme density, the large release of lytic enzyme and the leakage of lysosomal enzyme[33-35]. The results of this study showed that compared with the data of age, gender, duration of diabetes, combined hyperlipidemia and combined hypertension in the two groups, the expression of urinary NAG and serum RBP in group B was higher than that in group A, suggesting that the expression of serum RBP and urinary NAG may be the cause of disease progression to DN in patients with T2DM.

In order to further verify the above conjecture, logistic regression model was used in this study. The results showed that urinary NAG and serum RBP were related to the occurrence of injury in diabetic patients. Urinary NAG and serum RBP overexpression may be risk factors of renal injury in T2DM patients, and ROC curve was drawn, the results showed that the AUC of urinary NAG and serum RBP expression alone and in combination in predicting DN was > 0.80, and the predictive value was satisfactory, suggesting that urinary NAG and serum RBP overexpression are the key to lead to the progression of disease to DN in T2DM patients. The possible reasons for analysis may be: (1) When the renal tubules are damaged, the glomerular filtration decreased, the renal hemodynamics change, when the free RBP passes through the renal tubules, its ability to absorb and decompose the free RBP is limited, resulting in a large number of RBP retention, so the RBP in the serum shows a high expression state[36-38]; and (2) When the renal tubules degenerate, necrosis, damage and fall off, the NAG in the cells enters the urine with the exfoliated and necrotic cells, so a high level of NAG can be measured in the urine[39,40]. In addition, the pathways for obtaining urine NAG and serum RBP were relatively easy, the combination of urine NAG and serum RBP as early evaluation indicators of DN was based on two pathways of urine and blood, which was more reliable than the indicators in pure blood or urine. In this study, bivariate Spearman linear correlation analysis was also used, and the results showed that there was a positive correlation between urinary NAG and serum RBP expression in patients with DN, which may be due to the fact that both indicators are closely related to renal function, so the change of one of the indicators will certainly be cited another indicator changes, but the relationship between the two indicators lacks clinical demonstration support, and the reliability of the study needs to be further explored in the future.

CONCLUSION

In summary, elevated expression of urinary NAG and serum RBP may be risk factors leading to disease progression to DN in patients with T2DM, and the possibility of DN can be considered in patients with urinary NAG and serum RBP overexpression by examining urinary NAG and serum RBP expression in patients with T2DM in clinical practice.

ARTICLE HIGHLIGHTS

Research background

Diabetic nephropathy (DN) is a microangiopathy of type 2 diabetes mellitus (T2DM), which can damage the kidney through various ways and mechanisms due to the nature of the disease, involving the renal interstitium and glomeruli. However, in the early stage of the disease, patients only showed kidney volume increase and glomerular hyperthyroidism, and typical symptoms that are difficult to arouse individual attention were noticed. The symptoms were only noticed when the patients developed edema and proteinuria. At this time, the disease has progressed to an irreversible stage, and the best treatment timing should be taken. Therefore, finding new clinical biochemical factors or examination methods to help early detection of clinical DN patients is particularly important to guide the development of early intervention measures and improve the prognosis of patients.

Research motivation

This study provided new targets for early diagnosis and treatment of DN.

Research objectives

This study aimed to observe the expression of serum retinol-binding protein (RBP) and urinary N-acetyl-β-D-glucosaminidase (NAG) in patients with DN.

Research methods

Total 50 T2DM patients were retrospectively reviewed and included in group A. The baseline data of 50 patients with type 2 DN during the same period were collected and included in group B. The baseline data and serum RBP and urine NAG expression were compared between the two groups to analyze their value in the early prediction of DN.

Research results

The increased expression of urinary NAG and serum RBP may be the risk factors leading to the progression of T2DM to DN.

Research conclusions

The possibility of DN can be considered in patients with urinary NAG and serum RBP overexpression by examining the expression of urinary NAG and serum RBP in patients with T2DM in clinical practice.

Research perspectives

This study showed that urine NAG combined with serum RBP had good application prospects in the early detection of DN. Future studies can further expand the research sample size and improve the diagnostic accuracy of urinary NAG combined with serum RBP.

FOOTNOTES

ORCID number: Zhi-Hao Lin 0009-0003-7701-0282; Sheng-Feng Dai 0009-0003-1580-7392; Jian-Nan Zhao 0009-0000-7267-0968; Yue Jiang 0009-0002-5339-6398.

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Correlation between COVID-19 vaccination and diabetes mellitus: A systematic review

Yan-Fei He, Jing Ouyang, Xiao-Dong Hu, Ni Wu, Zhi-Gang Jiang, Ning Bian, Jie Wang

Yan-Fei He, Ni Wu, Ning Bian, Jie Wang, Health Management Center, The Sixth Medical Center, Chinese PLA General Hospital, Beijing 100048, China

Jing Ouyang, Casualty Management Section, The Sixth Medical Center, Chinese PLA General Hospital, Beijing 100048, China

Xiao-Dong Hu, Department of Endocrinology, The Sixth Medical Center, Chinese PLA General Hospital, Beijing 100048, China

Zhi-Gang Jiang, Department of Statistics, Zunyi Medical University, Zunyi 563006, Guizhou Province, China

Corresponding author: Yan-Fei He, MD, Associate Chief Physician, Doctor, Health Management Center, The Sixth Medical Center, Chinese PLA General Hospital, No. 6 Fu Cheng Road, Haidian District, Beijing 100048, China. heyanfeilc@163.com

Abstract

BACKGROUND
Coronavirus disease 2019 (COVID-19) is one of the current global public health threats and vaccination is the most effective tool to reduce the spread and decrease the severity of COVID-19. Diabetes is one of the important chronic diseases threatening human health and is a common comorbidity of COVID-19. What is the impact of diabetes on the immunization effect of COVID-19 vaccination? Conversely, does vaccination against COVID-19 exacerbate the severity of pre-existing diseases in patients with diabetes? There are limited and conflicting data on the interrelationship between diabetes and COVID-19 vaccination.

AIM
To explore the clinical factors and possible mechanisms underlying the interaction between COVID-19 vaccination and diabetes.

METHODS
We conducted a comprehensive search of PubMed, MEDLINE, EMBASE, and *Reference Citation Analysis* (https://www.referencecitationanalysis.com) online datab-ases, and medRxiv and bioRxiv gray literature using the keywords "SARS-CoV-2", "COVID-19", "vaccine", "vaccination", "antibody", and "diabetes" individually or in combination, with a cut-off date of December 2, 2022. We followed inclusion and exclusion criteria and after excluding duplicate public-

ations, studies with quantifiable evidence were included in the full-text review, plus three manually searched publications, resulting in 54 studies being included in this review.

RESULTS
A total of 54 studies were included, from 17 countries. There were no randomized controlled studies. The largest sample size was 350963. The youngest of the included samples was 5 years old and the oldest was 98 years old. The included population included the general population and also some special populations with pediatric diabetes, hemodialysis, solid organ transplantation, and autoimmune diseases. The earliest study began in November 2020. Thirty studies discussed the effect of diabetes on vaccination, with the majority indicating that diabetes reduces the response to COVID-19 vaccination. The other 24 studies were on the effect of vaccination on diabetes, which included 18 case reports/series. Most of the studies concluded that COVID-19 vaccination had a risk of causing elevated blood glucose. A total of 12 of the 54 included studies indicated a "no effect" relationship between diabetes and vaccination.

CONCLUSION
There is a complex relationship between vaccination and diabetes with a bidirectional effect. Vaccination may contribute to the risk of worsening blood glucose in diabetic patients and diabetic patients may have a lower antibody response after vaccination than the general population.

Key Words: COVID-19; Vaccination; Diabetes mellitus; Antibody; Blood glucose; Immune response

Core Tip: Coronavirus disease 2019 (COVID-19) is one of the current global public health threats and vaccination is the most effective tool to reduce the spread and decrease the severity of COVID-19. Diabetes is one of the important chronic diseases threatening human health and is a common comorbidity of COVID-19. There are limited and conflicting data on the interrelationship between diabetes and COVID-19 vaccination. Vaccination may be at risk of worsening glycemia in diabetic patients, and diabetic patients may have a lower immune response after vaccination than the general population, and there is a bidirectional relationship between vaccination and diabetes.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is one of the greatest public health threats to humanity in more than a century. The disease continues to rage across the globe, spanning countries and continents, with severe health, social and economic consequences for the world. COVID-19 is a multifactorial disease that affects nearly all organ systems in the body of the patient. Vaccination is one of the most effective tools to reduce transmission[1] and decrease clinical severity[2]. As of March 16, 2022, more than 10 billion different doses of the COVID-19 vaccine, including boosters, have been administered worldwide[3]. Diabetes mellitus (DM) is a chronic disease that causes high blood glucose levels due to failure of insulin secretion or action[4,5], affecting approximately 537 million adults[6]. DM remains one of the major risk factors for serious illness and worse outcomes in people with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection[7-9]. Many studies have shown that hyperglycemia is associated with an increase in the frequency and severity of any infection, not just COVID-19[10]. This raises concerns about the behavior of the COVID-19 vaccination in diabetic patients and the effects of having been vaccinated and the factors that influence it[11].

Reassuringly, the vaccine has demonstrated efficacy and safety in the prevention of severe COVID-19 in both phase III trials and real-world data[12-14]. The vaccine also plays a key role in protecting vulnerable populations associated with an increased risk of morbidity and mortality, including patients with diabetes[12]. However, there is evidence of multiple immunodeficiencies in patients with DM that affect the innate and acquired immune system[15]. Therefore, it can be expected that the protective effect of vaccination may be weaker compared to the general population. Previous studies have shown reduced immunogenicity to the hepatitis B vaccine in patients with DM, while results are less consistent for influenza, pneumococcal, and varicella zoster[16]. In several recent studies using real-world data,

vaccine efficacy was found to be lower in patients with DM than in the total population[17,18], while another Japanese study reported no significant association between vaccine efficacy and DM[19]. There are conflicting results regarding the immune efficacy of the COVID-19 vaccine in patients with DM. Furthermore, hyperglycemic crisis, acute myocardial injury[20], Guillain-Barre syndrome[21], and herpes zoster[22] are some of the very rare vaccine-related adverse events that have been reported occasionally. In patients with pre-existing DM, does the COVID-19 vaccination cause perturbations in blood glucose levels or even alter the natural history of the disease? There are very limited data on the interrelationship between DM and COVID-19 vaccination.

Therefore it seems important and interesting to understand the interrelationship between COVID-19 vaccination and diabetes. To elucidate this complexity, we summarized almost all current clinical studies and systematically analyzed various factors regarding the interconnection between DM and COVID-19 vaccination in order to inform diabetic patients of the optimal vaccination strategy and clinical management.

MATERIALS AND METHODS

Identify research question
What is the effect of DM on the immunization effect of COVID-19 vaccination? Conversely, does vaccination against COVID-19 disrupt blood glucose? Or accelerate the progression of pre-existing diabetic complications?

Identify relevant types of evidence
An experienced information specialist conducted a comprehensive search of PubMed, MEDLINE, and EMBASE online databases with no time limit, and the last data update was December 2, 2022. We used the keywords "SARS-CoV-2", "COVID-19", "vaccine", "vaccination", "antibody", and "diabetes" individually or in combination to achieve a comprehensive literature search. We also searched the gray literature of medRxiv and bioRxiv as well as the most recent literature of the *Reference Citation Analysis* (https://www.referencecitationanalysis.com). Finally, we manually searched the references cited in the original articles included in the study in order to avoid missing any relevant and important literature. Inclusion criteria were all studies conducted in humans that discussed the relationship between DM and vaccination against COVID-19. Studies that included the same population but reported different data and outcomes were also included. Exclusion criteria were: Non-human (animal), non-English, only exploring willingness to vaccinate, and participants who were not diabetic or who received a vaccine other than the COVID-19 vaccine. The type of diabetes, the type of vaccine, the age of participants, and the type of literature were not restricted. A detailed search strategy is available in the Supplementary Material.

Study selection
After completing the initial search, two independent reviewers conducted a screening process, and literature with quantifiable evidence was included in our review, including case reports, qualitative analyses, and other gray literature. We excluded repetitive publications and articles without relevant data. One reviewer reviewed the selected articles in their entirety, and studies containing full data descriptions were used for data graphs. Any conflicts that arose during the data extraction process were discussed or consulted and resolved by third-party experts. All seven authors were involved in the discussions. Figure 1 shows a visual representation of the inclusion workflow.

Data charting
A total of 2142 publications were retrieved as of December 2, 2022, and after screening by the inclusion criteria described above, we reviewed 208 full-text papers for eligibility, plus three manually retrieved papers, resulting in 54 papers included in this review (Figure 1). We extracted data for each paper regarding the first author's name, country, study design, basic demographic characteristics of participants, the type of vaccination, vaccination regimen, and blood glucose for tabulation and discussion. We did not perform any meta-analysis of the data obtained because, as expected, there was substantial heterogeneity among the designs, methods, populations, and vaccines used in the studies we encountered, making meaningful comparisons between studies impossible. A summary of information on the included studies is presented in Table 1.

RESULTS

A total of 54 studies were included[18,23-75], from 17 countries, including 9 from Japan. The earliest date of the studies was November 2020[48]. There were no randomized controlled studies, but two studies applied propensity score matching (PSM) methods. What was surprising was that one study

Table 1 Characteristics of the included studies

Ref.	Country	Study design	Study time span	Population	Sample size (*n*)	No. of patients with DM (*n*) T1DM T2DM		Sex (F/M)	Age, median (min-max), yr	Type and name of vaccine	Dose schedule	Related findings
Zhang *et al*[23]	China	Observational study	Between October 2021 and January 2022	The population is aged ≥ 60 yr with hypertension or (/and) DM	1413	620		661/752	67.6	Vero cell (19nCov-CDC-Tan-HB02)	Two doses (day 0, day 28)	After vaccination, there was no significant abnormal fluctuation in blood glucose in diabetic patients
Marfella *et al*[24]	Italy	Prospective observational study	December 2020	Healthcare and educator workers	478		201	212/266	18-60	mRNA-BNT162b2 (Pfizer-BioNTech) or ChAdOx1-S (Astra-Zeneca) or mRNA-1273 (Moderna)	One (day 0, day 21) or two (day 52) doses	Significant decrease in the immune response in people with poorly controlled blood glucose
Kılınç-Toker *et al* [25]	Turkey	Retrospective study	Between August 1, 2021 and October 31, 2021	Hospitalized patients with COVID-19	541	195		282/259	70.2 (21-98)	(CoronaVac) and/or BNT162b2 mRNA (Pfizer-BioNTech)	14 d after dose 2	For hospitalized patients after the second dose, diabetes was not associated with their ICU stay and mortality
Barocci *et al*[26]	Italy	Observational study	Between December 2020 and June 2021	Healthcare workers and university staff	284[5]	8		155/129	43-61	ChAdOx1-S and (BNT162b2/BNT162b2 and ChAdOx1-S/ChAdOx1-S)	2 mo after dose 2	DM does not affect antibody levels
Singh *et al*[27][1]	India	Cross-sectional study	Between January 16, 2021 and May 15, 2021	Healthcare workers	515[4]	0	52	210/305	44.8 ± 13.1[9]	Covishield™ (ChAdOx1-nCOV) or Covaxin™ (BBV-152)	One (day 21) and two (day 21-28, day 83-97, and day 173-187) doses	People with T2DM had a significantly lower seropositivity rate compared to those without
Singh *et al*[28][1]	India	Longitudinal study	Between January 16, 2021 and November 15, 2021	Healthcare workers	481	0	51	195/286	≤ 60 years, *n* = 411; > 60 years, *n* = 70	Covishield™ (ChAdOx1-nCOV) or Covaxin™ (BBV-152)	3 wk, 3 mo, and 6 mo after dose 2	Participants with T2DM have a lower seropositivity rate at all time points
Shim *et al*[29]	Korea	Retrospective study	February2021	Vaccination participants	736	48		433/303	51.5 (20-80)	AZD1222, BNT162b2, mRNA-1273 and Ad26.COV2.S	2 wk before and 6 mo after dose 2	Diabetics had a lower rate of neutralizing antibodies after vaccination
Alqassieh *et al*[30]	Jordan	Prospective observational cohort	Between March and April 2021	Jordanian adults	288	76		189/151	20-60 years, *n* = 137, > 60 years, *n* = 151	Pfizer-BioNTech or Sinopharm	6 wk after dose 2	Although DM negatively affected IgG titer, it was not statistically significant
Wan *et al*[31]	China (Hong Kong)	Population-based study	Between February 23, 2021 and	Patients with T2DM in Hong Kong electronic case records	350963	0	350963	167073/183890	64.7 ± 1.37/68.1 ± 0.74[7]	BNT162b2 or CoronaVac	Complete at least one dose of vaccination	Patients with T2DM do not appear to have higher risks of AESI and acute diabetic

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Ref	Country	Study type	Study period	Population	Total			Sex (M/F)	Age	Vaccine	Timing	Conclusion
			January 31, 2022									complications after vaccination
Lee et al[32]	South Korea	Questionnaire study	Between March 8, 2021 and March 11, 2021	Healthcare workers	1603	27		1261/342	37.7 ± 10.8[9]	ChAdOx1	7 d after dose 1	DM is associated with an increased risk of grade 3 to 4 adverse reactions after the first dose
Rangsrisaeneepitak et al[33]	Thailand	PSM observational study	Between June 8, 2021 and July 12, 2021	Healthcare workers and T2DM patients	282		94	129/153	30-83	ChAdOx1 nCoV-19 (AZD1222)	56 d after dose 1	People with T2DM had weaker antibody responses than those without diabetes after the first dose
Sourij et al[34]	Austria	Multicentre prospective cohort study	Between April and June 2021	T1DM, T2DM, and healthy participants	150	75	75	68/82	49.2 ± 14.5[9]	BioNTech-Pfizer, Moderna, or AstraZeneca	7 to 14 d after dose 1 and 14 to 21 d after dose 2	The antibody levels after the second vaccination were comparable in healthy controls and DM patients, irrespective of glycaemic control
Tawinprai et al[35]	Thailand	Prospective cohort study	Between March 31, 2021 and May 5, 2021	Healthcare workers	796	11		517/279	40 (30-57)[3]	ChAdOx1 (AZD1222)	At least 21 d after dose 1 and before dose 2	DM reduces the immune response to vaccination
Ali et al[18]	Kuwait	Case-control study	August 2021	Non-diabetics and patients with T2DM	262	0	81	126/136	49.3 ± 14.5[9]	BNT162b2 (Pfizer-BioNTech)	At least 3 wk after dose 2	Both neutralizing antibody and IgG antibody titers were significantly lower in the T2DM group than in the non-diabetic group
Karamese et al[36]	Turkey	Descriptive study	March 2021	Participants over 65 years of age who have received two doses of vaccine	235	49		111/124	70.4 ± 4.8[9]	CoronaVac	4 wk after dose 1 and 4 wk after dose 2	Lower rates of antibody response were detected in participants with DM
Lustig et al[37]	Israel	Single-centre, prospective, longitudinal cohort study	Between December 19, 2020 and January 30, 2021	Health-care workers	2607	139		1883/724	47.7 ± 12.5[9]	Pfizer-BioNTech BNT162b2	1-2 wk after dose 1 and 1-2 wk after dose 2	Decreased antibody response in diabetic patients after vaccination
Islam et al[38]	Japan	Cross-sectional study	June 2021	Workers	953	21		654/299	21-75	BNT162b2 (Pfizer-BioNTech)	15 to 71 d after dose 2	Spike IgG antibody titers were lower in the presence of hyperglycemia
Parthymou et al[39]	Greece	Longitudinal observational cohort study	September 2021	Healthcare units participants	712	50		444/268	50.8 ± 11.4[9]	BNT162b2 (BioNTech-Pfizer)	3 wk and 3 mo after Dose2	DM is not an independent factor affecting antibody titers
Priddy et al[40]	New Zealand	Prospective cohort study	Between June 10, 2021 and September 18, 2021	Participants in two centers	285	28		156/129[6]	52 (16-92)	BNT162b2 (BioNTech-Pfizer)	28 d after dose 2	Participants with diabetes had lower anti-S IgG antibodies compared to those without DM
Naschitz et al[41]	Israel	Retrospective	May 2021	Residents in long-term	304	103		208/96	≥ 60	BNT162b2 (Pfizer-	3-4 mo after dose	DM is associated with

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Ref	Country	Study type	Period	Population					Age	Vaccine	Timing	Findings
		study		geriatric and palliative care and assisted living facilities						BioNTech)	2	negative serological results
Güzel *et al*[42]	Turkey	Prospective study	May 2021[2]	Volunteers, outpatient clinic people, and COVID-19 patients	183	80		98/85	21-60	CoronaVac-SinoVac	21 d after dose 2	IgG antibody levels were significantly lower in patients with DM than in those without DM
Virgilio *et al*[43]	Italy	Multicenter prospective study	Between June 2021 and December 2021	Residents of long-term care facilities	555	0	140	378/177	82.1	BNT162b2 (Cominarty) Moderna (mRNA-1273)	Before the vaccination, 2 mo, and 6 mo after dose 1	Vaccination in elderly residents with T2DM is associated with a reduced humoral immune response
Patalon *et al*[44]	Israel	Retrospective cohort study	Between February and May 2021	A large patient cohort from Maccabi Healthcare Services	4740	377		1914/2826	16-59 years, $n = 3355$; ≥ 60 years, $n = 1385$	BNT162b2 (BioNTech-Pfizer)	Two vaccinations at intervals of 21 to 27 d	DM is not a relevant factor affecting antibody levels
Mitsunaga *et al*[45]	Japan	Prospective study	Between April 15, 2021 and June 9, 2021	Hospital's workers	374	6		264/110	36	BNT162b2 vaccine (COMIRNATY (Tozinameran)	Before vaccination, 7 to 20 d after dose 1, and 7 to 20 d after dose 2	HbA1c higher than 6.5% was a significant suppressor of antibody responses
Papadokostaki *et al* [46]	Greece	Prospective observational study	Between May and September 2021.	Participants attended the vaccination center	174	14	44	107/67	52.6 ± 10.6	BNT162b2 (BioNTech-Pfizer)	21 d after dose 1, 7-15 d after dose 2, and 70-75 d after dose 2 but before dose 3	It was high and similar after the second dose in both participants with and without DM
Zhao *et al*[47]	United States	Prospective longitudinal study	Between December 2020 and December 2021	Veterans and healthcare workers	124	39		33/91	20-95	BNT162b2 (Pfizer-BioNTech)	48 h before dose 1 and dose 2, 1 mo, 3 mo, 6 mo, 12 mo after dose 2, and 1 mo after dose 3	DM was significantly associated with a decrease in response intensity after completion of the primary vaccine series, but responses to the third dose were generally robust
Santotoribio *et al* [48]	Spain	Descriptive, retrospective, observational, and cross-sectional study	Between November 1, 2020 and March 31, 2021	Infected patients and vaccinated subjects	175	17		112/63	51.0 (19-89)	Pfizer-BioNTech	At least 21 d after dose 2	Serum antibody levels did not decrease significantly in patients with DM
Mehta *et al*[49]	India	Observational cohort study	Between March 2021 and October 2021	Vaccinated patients with AIRDs	495	63		416/79	56.5	AZD1222 (AstraZeneca)	4 wk and 10-14 wk after dose 2	DM was significantly associated with lower anti-RBD antibodies
Ajlan *et al*[50]	Saudi Arabia	PSM prospective study	June 14, 2022[2]	Patients from a large hospital	431	191		136/295	51.3 ± 16.2[9]	BNT162b2 or ChAdOx1	7 d after dose 1 and dose 2, and 2 wk after dose 1 and dose 2	There was no difference in the primary outcome between the two vaccine platforms. Unrespons-

												iveness was mainly linked to DM
Billany *et al*[51]	United Kingdom	Prospective observational study	March 2021	Maintenance hemodialysis patients	94	43		38/56	62.1 ± 12.2[9]	BNT162b2 or AZD1222	28 d after dose 1	There was no difference in antibody testing with or without DM
Aberer *et al*[52]	Austria	Multicenter prospective study	Between April and June 2021	DM patients	74	58	16	NR	T1DM: 39.5 ± 14.1; T2DM: 60.6 ± 6.2	BioNTech-Pfizer and Moderna and AstraZeneca	First dose	No change in insulin dose before and after vaccination. Vaccination significantly reduced TIR in T1DM patients, but had no effect on TIR in T2DM patients
Piccini *et al*[53]	Italy	Observational cohort study	Between March and June 2021	T1DM patients	39	39	0	17/22	18.7 ± 2.1[9]	mRNA-BNT162b1 (Pfizer-BioNTech) and Moderna (mRNA-1273)	One (day 7, day 14) and two (day 7, day 14) doses and 14 d after dose 1 and dose 2	COVID-19 vaccination was safe and not associated with significant perturbation of glycemic control in patients with T1DM
Heald *et al*[54][1]	United Kingdom	Observational cohort study	Between January 14, and March 7, 2021	T1DM patients	20	20	0	11/9	53 (26-70)	mRNA-BNT162b2 (Pfizer-BioNTech) and Oxford / AstraZeneca	7 d before and 7 d after dose 1	COVID-19 vaccination can cause temporary relative hyperglycemia in people with T1DM. No relationship between vaccine type and blood glucose perturbation
D'Onofrio *et al*[55]	Italy	Observational cohort study	July 13, 2021[2]	T1DM (AD) patients	35	35		14/21	36 (27-51)[3]	mRNA-BNT162b2 (Comirnaty)	14 d before and 3 d after dose 1 and dose 2	No significant differences in TIR, TAR, TBR, and CV between, after, and before the COVID-19 vaccination in T1DM patients
Heald *et al*[56][1]	United Kingdom	Survey and evaluation study	Between January 5, 2021 and April 4, 2021	Adults (18 years of age or more) with T1DM	97	97	0	51/46	44 (18-70)	Pfizer-BioNTech or Oxford-AstraZeneca	7 d before and 7 d after dose 1	In T1DM, vaccination can cause a temporary perturbation of interstitial glucose. There is no difference between vaccines
Gouda *et al*[57]	Greece	Observational study	March 2022	T1DM patients	135[8]	135	0	72/63	11.7 (5-18)	BNT162b2 (Pfizer-BioNTech), Moderna (mRNA-1273), or AstraZeneca	7 d before and 7 d after dose 1, dose 2, and dose 3	SARS-CoV-2 vaccination in children and adolescents with T1DM is safe and is not associated with immediate glucose imbalance
Sakurai *et al*[58]	Japan	Case report	December 11, 2021[2]	Healthy woman	1			1/0	36	mRNA-BNT162b2 (Pfizer-BioNTech)	First dose	mRNA vaccine is associated with new-onset T1DM
Patrizio *et al*[59]	Italy	Case report	September 15, 2021[2]	T2DM patient	1	0	1	0/1	52	mRNA-BNT162b2 (Pfizer-BioNTech)	Second dose	T1DM may be triggered after SARS-CoV-2

Ref.	Country	Study type	Date	Patient description	No.			M/F	Age	Vaccine	Dose	Conclusion
												vaccination
Aydoğan *et al*[60]	Turkey	Case series	Between May 2021 and October 2021	One had Hashimoto's thyroiditis, and the other 3 were healthy	4			1/3	27-56	mRNA-BNT162b2 (Pfizer-BioNTech) or CoronaVac	Second dose	Vaccination with BNT162b2 may trigger T1DM
Sato *et al*[61]	Japan	Case report	April 19, 2022[2]	Malignant melanoma patient	1			0/1	43	mRNA-based SARS-CoV-2 vaccination	Second dose	mRNA vaccine may trigger T1DM
Yakou *et al*[62]	Japan	Case series	December 21, 2021[2]	T1DM patients	2	2	0	2/0	52-71	mRNA-BNT162b2 (Pfizer-BioNTech)	Second dose	A temporary decrease in insulin secretion after vaccination
Mishra *et al*[63]	India	Case series	Between January 18, 2021 and March 4, 2021	T2DM patients	3	0	3	1/2	58-65	Covishield™ (ChAdOx1-nCOV) (AstraZeneca)	First dose	Vaccination may result in a mild and temporary increase in blood glucose levels
Abu-Rumaileh *et al* [64]	Jordan	Case report	January 14, 2021	Hypertension patient	1			0/1	58	mRNA-BNT162b1 (Pfizer-BioNTech)	Second dose	COVID-19 vaccine has a risk of causing new-onset T2DM
Sasaki *et al*[65]	Japan	Case report	December 13, 2021[2]	Osteoporosis, mild glucose intolerance	1	0	0	1/0	73	Moderna (Spikevax, mRNA-1273)	Second dose	The development of T1DM is attributable to the COVID-19 vaccination
Lee *et al*[66]	United States	Case Series	June 30, 2021[2]	T2DM and hypertension patients	3	0	2	1/2	52-87	mRNA-BNT162b1 (Pfizer-BioNTech) and Moderna (Spikevax, mRNA-1273)	First dose	Vaccination may trigger a hyperglycemic episode and DKA
Edwards *et al*[67]	United Kingdom	Case Series	April 2021	Hypertension, hypothyroidism, and pre-diabetes	3			0/3	53-68	Covishield™ (ChAdOx1-nCOV)	First dose	The first administration of the COVID-19 vaccine can trigger an acute hyperglycemic crisis
Ganakumar *et al* [68]	India	Case series	November 2021	T1DM	2	2	0	1/1	20-25	COVISHIELD (ChAdOx1 nCoV-19) or COVAXIN (BBV152)	1 to 4 d after dose 2	COVID-19 Vaccination has the potential to induce DKA
Zilbermint *et al*[69]	United States	Case report	September 11, 2021[2]	T1DM	1	1	0	1/0	24	Moderna (mRNA-1273)	15 h after dose 2	A plausible mechanism exists between COVID-19 vaccination and DKA
Yaturu *et al*[70]	United States	Case report	May 2021	Hypertension, primary hyperparathyroidism, and obesity patient	1	0	1	0/1	56	BNT162b2 (Pfizer-BioNTech)	Right after the second dose	COVID-19 Vaccination has the potential to induce HHS
Kshetree *et al*[71]	United States	Case report	NR	Hypertension and pre-diabetes	1	1	0	0/1	69	mRNA vaccine	2 mo after dose 3	COVID-19 mRNA vaccine has the potential to induce DKA
Prasad[72]	India	Case report	March 2021	Patient with T2DM	1	0	1	1/0	73	Covishield	6 d after dose 1	Vaccination may cause glycaemic disturbances

Sasaki *et al*[73]	Japan	Case report	January 4, 2022[2]	Healthy person	1	1	0	1/0	45	BNT162b2 (Pfizer-BioNTech)	1 d after dose 1	COVID-19 vaccine might trigger the onset of fulminant T1DM in susceptible individuals
Yano *et al*[74]	Japan	Case report	November 11, 2021[2]	Healthy person	1	1	0	1/0	51	Moderna (mRNA-1273)	28 d after dose 1	COVID-19 vaccination can induce T1DM in some individuals
Ohuchi *et al*[75]	Japan	Case report	November 2021[2]	Cutaneous malignant melanoma with axillary lymph node metastasis	1	1	0	0/1	45	BNT162b2 (Pfizer-BioNTech)	3 d after dose 2	There is a highly suspicious causal relationship between fulminant T1DM and COVID-19 vaccination

[1]The authors are the same, but the individual studies are different, including different phases, different samples, and different data.

[2]Take the date of receipt of the manuscript.

[3]Median (25th-75th percentile).

[4]Sample size for completing the second dose.

[5]Sample size for fully completed questionnaires.

[6]Contains a Non-binary participant.

[7]Age (mean ± SD) is divided according to BNT162b2 and CoronaVac groups.

[8]Sample size for T1DM, of which 70 received at least one dose of the vaccine and the other 65 were unvaccinated.

[9]mean ± SD.

NA: Not available; NR: Not reported; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; PSM: Propensity score matching; HbA1c: Glycated hemoglobin; TIR: Time in range; DM: Diabetes mellitus; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; HHS: Hyperosmolar hyperglycemic syndrome; DKA: Diabetic ketoacidosis; AD: Autoimmune diabetes; AIRDs: Autoimmune Rheumatic Diseases; AESI: Adverse events of special interest; F: Female; M: Male; ICU: Intensive care unit; TAR: Time above range; TBR: Time below range; CV: Coefficient variation.

analyzed the bidirectional relationship between vaccination and blood glucose[23]. There were 30 studies that discussed the effect of diabetes on vaccination[18,23-51], two of which were specifically about whether DM increased adverse effects after vaccination[31,32], and three of which had participants with autoimmune rheumatic disease[49], organ transplantation[50], and a special group on blood pressure dialysis[51]. The other 24 studies were on the effect of vaccination on DM[52-75] and included 18 case reports or case series[58-75]. The largest sample size was 350,963, a population-based study from Hong Kong, China, which evaluated the risk of adverse events of special concern and acute diabetic complications after COVID-19 vaccination in the type 2 DM (T2DM) population[31]. Of the sample included in the 54 studies, the youngest age was five years[57] and the oldest was 98 years[25]. Only one study analyzed the effects of glycemia on both cellular and humoral responses after vaccination[24]. Only one study performed a comparative analysis between type 1 diabetes and type 2 diabetes[34]. The authors of some studies claim that they are reporting for the first time, trying to fill a gap in the literature regarding certain relationships between COVID-19 vaccination and DM.

Results on the effect of vaccination on DM

From the current studies, the effect of vaccination on diabetes is mainly manifested in the effect on blood glucose after vaccination, with a total of 24 studies describing this relationship, including 18 case reports or case series. To make the various characteristics of these case series readily apparent, we have
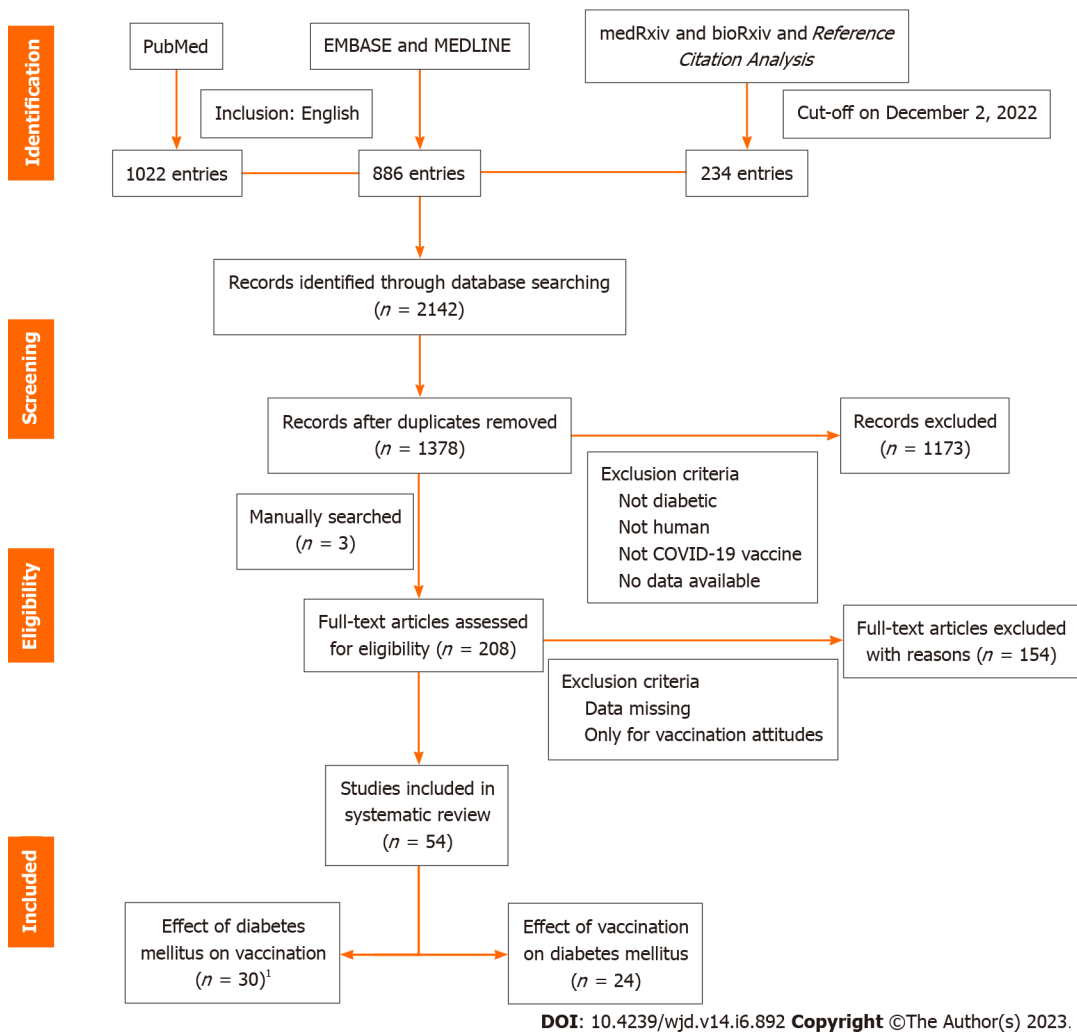
Figure 1 Flow diagram of literature search. [1]One study analyzed the bidirectional relationship between vaccination and blood glucose. COVID-19: Coronavirus disease 2019.

additionally tabulated a total of 29 cases from these 18 case reports or case series (Table 2). Of these 29 cases, 12 were new-onset type 1 DM (T1DM) and three were new-onset T2DM. Fourteen cases were vaccinated with two doses, 14 with only one dose, and one with a third dose. mRNA vaccines were used in 19 cases (13 cases of mRNA-BNT162b2 (Pfizer-BioNTech) and 6 cases of Moderna (mRNA- 1273)) and eight cases used the adenoviral vector vaccine Covishield™ (ChAdOx1-nCOV or AstraZeneca). Most events occurred within days of vaccination, with the longest being a diagnosis of new-onset T1DM two months after the third dose[71]. No deaths were reported. Of these 24 studies, only three indicated that vaccination had no effect on blood glucose[53,55,57], while the rest indicated that it may cause an increase in blood glucose. No vaccinated individuals with episodes of hypoglycemia were identified. Of course, it cannot be ruled out that some patients develop mild or self-limiting hypoglycemia after vaccination, which may not cause certain subjective symptoms in patients and therefore may go undocumented by clinical diagnosis.

Results on the effect of DM on vaccination

Of the 30 studies on the effect of DM on vaccination, only one study analyzed the correlation between blood glucose levels and the humoral and cellular immunity of the organism after immunization[24]. Most of the studies examined whether blood glucose levels as an indicator of effect or DM as comorbidity negatively affected the immune response to vaccination. Twenty-one of the studies showed that DM reduced response to vaccination, while the other nine indicated that DM had no effect on vaccine efficiency[23,25,26,30,34,44,46,48,51]. Some studies also quantified the association with vaccine biological effects in terms of patient-specific attributes. Fifteen studies expressed a negative correlation between age and immune response, with older individuals having a weaker immune response than their younger individuals[25,28-30,32-34,36,37,40,42,45,47,51]. Seven studies showed a correlation between gender and immune response after vaccination, with women having a more positive immune effect than men[25,27,32,33,35,39,44]. Eight studies analyzed the effect of vaccine type on the immune

Table 2 Summary of the case report or case series about the effect of SARS-CoV-2 vaccination on blood glucose

Ref.	Age (yr)	Gender	Type and name of vaccine	Blood glucose (mg/dL)/HbA1c (%) pre-vaccination post-vaccination		Onset after vaccination	Pre-existing condition	Final diagnosis	C-peptide (ng/mL)	GAD65Ab (IU/mL)	Treatment	Outcomes	Conclusion
Sakurai *et al* [58]	36	Female	mRNA-BNT162b2 (Pfizer-BioNTech)	Normal	501/7.0	3 d after dose 1	None	Fulminant T1DM	0.13	NA	Insulin infusion	Discharged	mRNA vaccine is associated with new-onset T1DM
Patrizio *et al* [59]	52	Male	mRNA-BNT162b2 (Pfizer-BioNTech)	53[1]	87[1]	4 wk after dose 2	Vitiligo vulgaris and T2DM	Graves' disease and T1DM	1	61.2	Insulin analogues	NR	T1DM may be triggered after SARS-CoV-2 vaccination
Aydoğan *et al*[60]	56	Male	mRNA-BNT162b1 (Pfizer-BioNTech)	Normal	440/8.2	15 d after dose 2	Vitiligo vulgaris and Hashimoto's thyroiditis	T1DM	1.5	> 2000	Insulin infusion	Recovery	Vaccination with BNT162b2 may trigger T1DM
	48	Male	mRNA-BNT162b2 (Pfizer-BioNTech)	Normal	352/10.1	8 wk after dose 2	None	T1DM	0.97	94	Low-carbohydrate diet	Recovery	
	27	Male	mRNA-BNT162b2 (Pfizer-BioNTech)	Normal	320/12.5	3 wk after dose 2	None	T1DM	0.87	725	Basal insulin	Recovery	
	36	Male	mRNA-BNT162b2 (Pfizer-BioNTech) and CoronaVac	Normal	526/12.6	3 wk after dose 2	None	T1DM	0.38	234	Insulin infusion	Recovery	
Sato *et al*[61]	43	Male	mRNA-based SARS-CoV-2 vaccination	94/5.6	655/8.0	14 d after dose 2	Malignant melanoma	Fulminant T1DM	0.33		Insulin infusion	Discharged	mRNA vaccine may trigger T1DM
Yakou *et al* [62]	71	Female	mRNA-BNT162b1 (Pfizer-BioNTech)	93/8.1	944/8.0	1 d after dose 2	T1DM	Diabetic ketoacidosis	< 0.03	> 2000	Insulin infusion	Discharged	Risk of inducing ketoacidosis after vaccination in T1DM patients
	52	Female	mRNA-BNT162b1 (Pfizer-BioNTech)	106	494/11.6	1 d after dose 2	T1DM	Diabetic ketoacidosis	ND	123	Insulin infusion	Discharged	
Mishra *et al* [63]	58	Female	Covishield™ (ChAdOx1-nCOV) (AstraZeneca)	110	183	1 d after dose 1	T2DM	T2DM	NR	NR	Increased dose of metformin.	Discharged	Vaccination may result in a mild and temporary increase in blood glucose levels
	64	Male	Covishield™ (ChAdOx1-nCOV) (AstraZeneca)	95	150	1 d after dose 1	T2DM	T2DM	NR	NR	Without additional intervention	Discharged	
	65	Male	Covishield™ (ChAdOx1-nCOV) (AstraZeneca)	107	186	6 d after dose 1	T2DM	T2DM	NR	NR	Without additional intervention	Discharged	
Abu-Rumaileh *et al*[64]	58	Male	mRNA-BNT162b1 (Pfizer-BioNTech)	80	1253/13	26 d after dose 1	Hypertension	T2DM	1.1	NR	Insulin infusion	Discharged	COVID-19 vaccine has a risk of causing new-onset T2DM

Sasaki *et al*[65]	73	Female	Moderna (Spikevax, mRNA-1273)	7.3	318/9.3	8 wk after dose 2	Osteoporosis, mild glucose intolerance	T1DM	0.48	> 2000	Intensive insulin therapy	NR	COVID-19 Vaccination may lead to the new-onset T1DM
Lee *et al*[66]	52	Female	mRNA-BNT162b2 (Pfizer-BioNTech)	5.5-6.2	1062/12.0	3 d after dose 1	Hypertension	T2DM and nonketotic HHS	NR	NR	Insulin infusion.	Discharged	Vaccination may trigger HHS
	60	Male	Moderna (mRNA-1273)	7.5	847/13.2	2 d after dose 1	T2DM	T2DM and HHS	NR	NR	Insulin infusion	Discharged	Vaccination may trigger a hyperglycemic episode
	87	Male	Moderna (mRNA-1273)	7	923	10 d after dose 1	T2DM	T2DM and HHS and DKA	NR	NR	Insulin infusion	Discharged	Vaccination may trigger HHS and DKA
Edwards *et al*[67]	59	Male	Covishield™ (ChAdOx1-nCOV)	5.6	594/14.1	21 d after dose 1	Obesity	Hyperglycemic ketosis	235[2]	NR	NA	Discharged	The first administration of the adenovirus-vectored COVID-19 vaccine can trigger an acute hyperglycemic crisis
	68	Male	Covishield™ (ChAdOx1-nCOV)	6.5	918/14.7	36 d after dose 1	Pre-diabetes	Mixed HHS/DKA	561[2]	NR	ICU admission	Discharged	
	53	Male	Covishield™ (ChAdOx1-nCOV)	6.2	576/17.1	20 d after dose 1	Pre-diabetes	DKA	377[2]	NR	ICU admission	Discharged	
Ganakumar *et al*[68]	20	Male	COVISHIELD (ChAdOx1 nCoV-19)	NR	14.1	1 d after dose 2.	None	Severe DKA	NR	NR	Insulin infusion	Discharged	COVID-19 vaccination has the potential to induce DKA
	25	Female	COVAXIN (BBV152)	NR	16.3	4 d after dose 2	None	Severe DKA	NR	NR	Insulin infusion	Discharged	
Zilbermint *et al*[69]	24	Female	Moderna (mRNA-1273)	NR	505/12.0	15 h after dose 2	T1DM	Severe DKA	NR	NR	Insulin infusion	NR	A plausible mechanism exists between COVID-19 vaccination and DKA
Yaturu *et al*[70]	56	Male	BNT162b2 (Pfizer-BioNTech)	5.6	997/14	Right after the second dose.	Hypertension, primary hyperparathyroidism, and obesity	T2DM and HHS	NR	NR	Insulin infusion	Discharged	COVID-19 vaccination has the potential to induce HHS
Kshetree *et al*[71]	69	Male	mRNA vaccine	5.8	13.7	Two months after dose 3	Hypertension and pre-diabetes	T1DM and DKA	0.4	0.33	Insulin infusion	Discharged	COVID-19 mRNA vaccine has the potential to induce DKA
Prasad[72]	73	Male	Covishield	92/7.1	215/8	6 d after dose 1	T2DM	T2DM	NR	NR	Insulin infusion	Discharged	Vaccination may cause glycaemic disturbances
Sasaki *et al*[73]	45	Female	BNT162b2 (Pfizer-BioNTech)	Normal	344/7.6	1 d after dose 1	None	Fulminant T1DM and DKA	NR	NA	Insulin infusion	Discharged	COVID-19 vaccine might trigger the onset of fulminant T1DM in susceptible individuals
Yano *et al*[74]	51	Female	Moderna (mRNA-1273)	Normal	648/10.3	28 d after dose 1	None	Fulminant T1DM and DKA	1.72	NA	Insulin infusion	Discharged	COVID-19 vaccination can induce T1DM in some individuals
Ohuchi *et al*	45	Male	BNT162b2 (Pfizer-	NR	655	3 d after dose	Cutaneous malignant	Fulminant	0.99	Negative	NR	NR	There is a highly suspicious

[75]	BioNTech)	2	melanoma	T1DM	causal relationship between fulminant T1DM and vaccination, especially in patients treated with ICI

[1]Unit: mmol/mol and reference range is 20-38.

[2]Unit: pmol/L and the reference range is 370-1470.

NA: Not available; ND: Not detected; NR: Not reported; COVID-19: Coronavirus disease2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; HbA1c: Glycated hemoglobin; DM: Diabetes mellitus; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; HHS: Hyperosmolar hyperglycemic syndrome; DKA: Diabetic ketoacidosis; ICI: Immune checkpoint inhibitors.

response after vaccination in patients with DM, and four of these studies showed an effect[26,27,30,50]. There were also studies that concluded that mixed or heterologous vaccination produced better vaccine efficiency[25,26]. Three studies suggested that participants with previous SARS-CoV-2 infection would have a better antibody response than SARS-CoV-2-naive individuals[28,47,51]. We attempted to systematize the variables in the literature regarding the interrelationship between diabetes and vaccination and summarized the important findings of the studies related to these variables in Table 3. Ten studies mentioned adverse effects of vaccination[23,26-29,33-35,50,53] and only one study manifested that it would have an effect on antibody production[29]. Regarding the effect of BMI on vaccination, one study stated that a lower BMI increased the risk of grade 3 to 4 adverse reactions compared to normal-weight individuals[32], while another study showed that a higher BMI decreased the immune response after vaccination[42].

Results for "no effect"

Of the 54 studies included, a total of 12 studies indicated a "no effect" relationship between DM and vaccination. Nine of them concluded that DM had no effect on the immune response to the vaccine[23, 25,26,30,34,44,46,48,51]. Similarly, three studies showed no effect of vaccination on DM or blood glucose [53,55,57]. Of the two studies that specifically investigated DM and adverse reactions to vaccination[31, 32], one suggested that patients with T2DM did not appear to have a higher risk of adverse reactions after vaccination[31].

DISCUSSION

Effect of the COVID-19 vaccination on DM

Does COVID-19 vaccination lead to dysglycemia or even a hyperglycemic crisis with serious adverse consequences in patients? Of the 54 studies included, most suggested that there may be some association between vaccination and blood glucose, mainly in the form of elevated blood glucose or even induction of new-onset DM. Table 2 Lists 12 cases of new-onset DM. In addition, Heald *et al*[54] also implied that COVID-19 vaccination can cause temporary relative hyperglycemia in patients with T1DM. SARS-CoV-2 infection is known to cause an immune stress response and dysglycemia. The worsening of blood glucose that occurs after vaccination is thought to have a possible common pathophysiology with the hyperglycemia associated with SARS-CoV-2 infection. Possible mechanisms

Table 3 Outcomes of the studies based on the association between vaccination and diabetes

Ref.	Assessed variables	Findings related to variables	Conclusion	Limitations
Zhang *et al*[23]	Hypertension, Comorbidity, Side effects	None	After vaccination, no significant abnormal fluctuations in blood glucose values were observed in the DM patients	Lack of data on the duration of antibodies after vaccination in the study population
Marfella *et al*[24]	HbA1c, Time since vaccination, type of vaccine	On Day 21 after the second vaccine dose, T2DM patients with HbA1c > 7% showed significantly reduced virus-neutralizing antibody capacity than normoglycemic subjects and T2DM patients with good glycaemic control. At 21 d after the first vaccine dose, neutralizing antibody titers and CD4 cytokine responses involving type 1 helper T cells were lower in T2DM patients with HbA1c levels > 7% than in individuals with HbA1c levels ≤ 7%. The reduction of HbA1c levels 52 d after vaccination was associated with neutralizing antibody titers and CD4 cytokine increases	Hyperglycemia at the time of vaccination can worsen the immune response, and proper glycemic control can improve the immune response	The statistical significance of the relevant indicators was relatively low
Kılınç-Toker *et al* [25]	Age, sex, mixed vaccination, delta variant, BMI, Diabetes, hypertension, COPD, cardiovascular diseases, chronic kidney disease, cancer	Age, male gender, delta variant, and mixed vaccination (CoronaVac plus BioNTech) were associated with death. The delta variant had higher ICU admission and mortality rate	For hospitalized patients who received two doses of the vaccine, diabetes was not associated with their ICU stay and mortality	Retrospective design, short follow-up, and assessment of inpatients only
Barocci *et al*[26]	Homologous vaccination, heterologous vaccination, type of vaccine, vaccine schedule, sex, age, BMI, smoking, DM, cardiovascular diseases, respiratory tract diseases, previous SARS-CoV-2 infection, side effects	Heterologous vaccination induced a significantly higher humoral response than homologous vaccination. The type of vaccine influenced antibody titers	DM does not affect antibody levels	Results were influenced by anti-S IgG levels in asymptomatic subjects
Singh *et al*[27][1]	Sex, T2DM, age, BMI, side effects, type of vaccine, dose 1, dose 2	Gender, presence of comorbidities, and vaccine type were independent predictors of antibody seropositivity and anti-spike antibody titer levels. Patients with T2DM had a significantly lower seropositivity rate compared to those without the comorbid disease. Seropositivity rates were lower in those with T2DM compared to those without T2DM. Both vaccine recipients had similar mild to moderate adverse events, and none had serious side effects	T2DM is associated with lower seropositivity rates and anti-spike antibody titers	No assessment of the cell-mediated immune response
Singh *et al*[28][1]	Age, previous SARS-CoV-2 infection, sex, BMI, side effects, type of vaccine, dose 1, dose 2, T2DM, blood group, dyslipidemia, ischemic heart disease	The seropositivity rate was significantly higher in the ≤ 60 years age group than in the > 60 years age group at all time points. GMT was significantly higher in participants with past SARS-CoV-2 infection than in SARS-CoV-2-naiveindividuals.	Participants with T2DM had a lower rate of seropositivity at all time points	The sample was drawn from a healthy population with few comorbidities
Shim *et al*[29]	Age, DM, type of vaccine, side effects, vaccination interval, hypertension, BMI, sex	There were significant differences in general and neutralizing antibodies based on age, vaccine type, vaccination interval, pain score, diabetes, and hypertension	For all vaccines, subjects with diabetes showed lower rates of neutralizing antibody production after vaccination	Vaccination priority policies bring hetero-geneity across age groups
Alqassieh *et al*[30]	Age, type of vaccine, hypertension, cardiovascular disease, DM, sex, BMI	Old people (> 60) had lower IgG titers than their younger counterparts. The use of the Pfizer-Biotech vaccine was positively associated with positive IgG titers, while cardiovascular disease had a negative effect on IgG titers. Although diabetes had a negative impact on positive IgG titers, it was not statistically significant	Although DM negatively affected IgG titer positivity, it was not statistically significant	Samples were collected only once at a specific period (6 wk) after vaccination
Wan *et al*[31]	Dose 1, dose 2, HbA1c, side effects	None	Patients with T2DM do not appear to have higher risks of AESI and acute diabetic complications after vaccination	Adverse events are defined using diagnosis codes and may be biased by underdiagnosis or misclassification

Lee *et al*[32]	Sex, age, DM, type of vaccine, BMI	Being young, female or underweight, and having diabetes were associated with an increased risk of developing grade 3 to 4 adverse reactions after the first dose of the ChAdOx1nCoV-19 vaccine	DM is associated with an increased risk of grade 3 to 4 adverse reactions after the first dose of vaccine, especially in women	Sample from relatively healthy subjects working in hospitals
Rangsrisaeneepitak *et al*[33]	T2DM, age, sex, BMI, side effects	After the first dose of AZD1222, the antibody response was weaker in T2DM patients than in non-diabetic patients. The seroconversion rate was higher in the control group than in the diabetic group. Older age was associated with a weaker antibody response in older diabetic patients. The GMC of SARS-CoV-2 IgG antibodies at 56 d was significantly lower in diabetic patients than in age- and sex-matched controls. In the age- and sex-matched controls, SARS-CoV-2 IgG antibody levels were significantly higher in women than in men. During the first 24 h, injection site reactions were more common in diabetic patients than in healthy controls	After the first dose of AZD1222, the antibody response was weaker in T2DM patients than in non-diabetic patients	Participants in the control group were healthcare workers, so natural immunity may have been a confounding factor
Sourij *et al*[34]	T2DM, eGFR, HbA1c, side effects, T1D	Age and renal function were significantly associated with the extent of antibody levels. The most common side effect was injection site reactions, with a significantly lower rate in patients with T2DM	The antibody levels after the second vaccination were comparable in healthy controls and in DM patients, irrespective of glycaemic control	Focused only on the humoral immune response after vaccination, but did not investigate the cellular immune response
Tawinprai *et al*[35]	DM, hematologic disease, sex, age, time since the first dose of vaccination, BMI, side effects, cardiovascular disease, hypertension, dyslipidemia, end-stage kidney disease	Participants with diabetes or hematologic comorbidities had lower concentrations of anti-RBD antibodies. Anti-RBD antibody concentrations were significantly higher in female participants than in male participants. The immune response was lower in older participants. Anti-RBD antibody concentrations were significantly higher at 2 and 3 mo post-vaccination than at 1-mo post-vaccination	Participants with diabetes or hematologic comorbidities had lower concentrations of anti-RBD antibodies	The presence of participants who did not complete two anti-RBD antibody assays withdrew from the study
Ali *et al*[18]	T2DM, age, sex, BMI, comorbidity, previous SARS-CoV-2 infection, hypertension	T2DM is associated with lower titers of neutralizing and IgG antibodies	Both neutralizing antibody and IgG antibody titers were significantly lower in the T2DM group than in the non-diabetic group	Participants in the study were self-selected verbally and through job advertisements
Karamese *et al*[36]	T2DM, age, hypertension, COPD, dose 1, dose 2	Lower antibody response rates were detected in participants with T2DM and in those aged 65 years and older	DM patients have lower antibody levels	The study population was an advanced age group with a high number of comorbidities
Lustig *et al*[37]	Age, sex, DM, immunosuppression, hypertension, heart disease, autoimmune disorders, BMI	Lower antibody concentrations are consistently associated with males, older age, immunosuppression, diabetes, hypertension, heart disease, and autoimmune disorders	Lower IgG concentrations and lower detectable IgA antibodies were observed in DM patients, indicating a reduced antibody response to vaccination in these patients	The sample was drawn from a healthy population with few comorbidities
Islam *et al*[38]	Hyperglycemia, FPG, age, sex, BMI, hypertension, smoking, alcohol consumption	Spike IgG antibody titers were lower in the presence of hyperglycemia and IFG	Vaccine recipients with diabetes and IFG had lower concentrations of SARS-CoV-2 spike IgG antibodies than the vaccine recipients with normoglycemia did	Associations observed in cross-sectional studies do not necessarily indicate causality
Parthymou *et al*[39]	Sex, age, smoking, BMI, DM, hypertension, statin use, vitamin D levels	Age, male gender, and tobacco use are negatively associated with antibody titers after COVID-19 vaccination	Antibody titers were numerically lower in diabetic patients, but this association was not statistically significant	Reliance on questionnaires to record anthropometric parameters and medical history affects reliability
Priddy *et al*[40]	Age, DM, sex, BMI, race	IgG and neutralization responses decreased with age. Lower responses were associated with age ≥ 75 and DM	Lower responses were associated with DM	Most of the IgG and neutralization tests used are not standardized
Naschitz *et al*[41]	Cancer, DM, congestive heart failure, sex, age,	Cancer, DM, or congestive heart failure were all associated with having a negative serology	DM is associated with negative serological	There was a large age difference between the

		result	results	two sample groups
	hypertension, COPD, cerebrovascular disease, chronic liver disease, cognitive disability			
Güzel *et al*[42]	Cardiovascular diseases, DM, age, BMI, sex, smoking, vitamin use, viral load, comorbidities	Cardiovascular disease and diabetes were associated with lower IgG antibody levels. In the healthcare workers group, IgG antibody response values were negatively correlated with BMI and age	IgG antibody levels were significantly lower in patients with DM than in those without DM	ELISA test may lead to false positive results
Virgilio *et al*[43]	Sex, T2DM, insulin therapy	The negative impact of diabetes in determining a steeper antibody decline was greater in female residents than in male residents. T2DM is associated with a reduced humoral immune response after SARS-CoV-2 vaccination. Antibody kinetics in diabetic patients receiving insulin therapy are similar to those in patients without diabetes	Vaccination in elderly residents with type 2 diabetes is associated with a reduced humoral immune response	Data on blood glucose or glycated hemoglobin levels were not specifically collected to assess the control or severity of diabetes
Patalon *et al*[44]	Sex, age, BMI, COPD, DM, congestive heart failure, inflammatory bowel disease	Females were associated with higher levels of antibodies. Lower antibody levels were observed in higher age groups	DM is not a relevant factor affecting antibody levels	The study population was older and had more comorbidities
Mitsunaga *et al*[45]	Age, Hypertension, HbA1c, Outdoor exercises, Vaccination interval, BMI, COPD, Dyslipidemia, DM, Autoimmune diseases, Cancer, dose 1, dose 2, BG	Older than 60 years, hypertension, HbA1c higher than 6.5%, and lack of outdoor exercises were significant suppressors of antibody responses, whereas the length of days from the first to the second vaccination longer than 25 d promoted a significant antibody response	HbA1c higher than 6.5% was a significant suppressor of antibody responses	The sample was relatively healthy health workers but did not include participants with serious comorbidities
Papadokostaki *et al* [46]	Age, DM, dose 1, dose 2, sample testing time, HbA1c, BMI, duration of diabetes, HbA1c	In the diabetic group, Abs-RBD-IgG was significantly correlated with age and time, and dose after vaccination	The humoral immune responses after the second dose were high and similar in participants with and without DM	No comparison between type 1 and type 2 diabetes
Zhao *et al*[47]	DM, dose 1, dose 2, dose 3, age, end-stage kidney disease, cancer, steroid use, previous SARS-CoV-2 infection, time since vaccination	DM was significantly associated with a decrease in response intensity after completion of the primary vaccine series, but responses to the third dose were generally robust. Age and malignancy had a negative effect on the initial strength of the humoral immune response. Being over 65 years, end-stage renal disease, diabetes, and clinical comorbidities of steroid use had a negative effect on the humoral immune response. SARS-CoV-2 infection enhanced the neutralization antibody response to the third dose	DM was significantly associated with a decrease in response intensity after completion of the primary vaccine series, but responses to the third dose were generally robust	Small sample size
Santotoribio *et al* [48]	Age, sex, DM, hypertension, heart disease	None	Serum antibody levels were not significantly reduced in patients with common conditions such as arterial hypertension, diabetes, heart disease, or chronic respiratory disease	No assessment of the cell-mediated immune response
Mehta *et al*[49]	DM, immunosuppression, vaccination interval, sex, comorbidity	DM, immunosuppression, and vaccination interval were all significantly associated with anti-RBD antibodies	DM patients had significantly lower titers of anti-spiking antibodies than patients without diabetes	The sample group was patients with autoimmune rheumatic diseases with a high proportion of comorbidities
Ajlan *et al*[50]	DM, type of vaccine, age, triple immunosuppressive therapy, side effects, sex, time since transplantation	Diabetes and triple immunosuppressive therapy appear to significantly affect the immune response. Triple immunosuppressive therapy and age were identified as significant factors in the lack of response to the vaccine after the second dose. Response rates after the first dose of vaccine with the Pfizer vaccine were higher than those with the AstraZeneca vaccine	Diabetes mellitus and triple immunosuppressive therapy appear to significantly affect response	Lack of immunocompetence control group
Billany *et al*[51]	Age, immunosuppression, previous SARS-CoV-2 infection, sex, race, DM	Patients with detectable antibodies were younger than patients without detectable antibodies. Patients who were immunosuppressed were less likely to have detectable antibodies than patients who were not	There was no difference in antibody testing with or without DM	Small sample size

		immunosuppressed. Patients previously infected with COVID-19 were more likely to have detectable antibodies than those with no history of SARS-CoV-2 infection		
Aberer *et al*[52]	TIR, TBR, TAR, T1DM, T2DM, carbohydrate intake, CV	None	At the time of side effects, T1DM patients had significantly less TIR and significantly more TAR, while there was no effect on T2DM patients	Short assessment time and small sample size
Piccini *et al*[53]	Side effects, dose 1, dose 2, TIR, time in different glucose ranges, mean glucose levels, TDD of insulin, bolus proportion, type of vaccine	Side effects after the vaccination were mild and more frequent after the second dose. No severe adverse reactions were reported	No significant differences in glycemic control and glycemic indices were observed at different times throughout the vaccination cycle and were independent of the vaccine type	Small sample size
Heald *et al*[54][1]	Age, BMI, mode of treatment, sex, HbA1c, type of vaccine, duration of diagnosed T1DM	The fall in the percentage BG on target was also greater for those with a median BMI of 28.1 kg/m^2 or more. The fall in the percentage BG on target categorized by additional Metformin/Dapagliflozin was greater than no oral hypoglycemic agents, and the median age ≥ 53 yr was greater than < 53 yr	In T1DM, COVID-19 vaccination can cause a temporary BG disturbance, and this effect is more pronounced in patients taking oral hypoglycemic drugs plus insulin and in the elderly	No analysis of changes in insulin dose in the week following the COVID-19 vaccination
D'Onofrio *et al*[55]	TIR, TBR, TAR, CV, dose 1, dose 2, insulin dosage, SD	None	Pre- and post-CGM data collected during the two vaccine doses did not show any significant differences between the two groups in terms of TIR, TAR, TBR, CV, and SD	Small sample size
Heald *et al*[56][1]	Medication, HbA1c, oral hypoglycemic drugs plus insulin therapy, age, sex, type of vaccine, duration with diabetes, BMI	COVID-19 vaccination can cause a temporary perturbation of interstitial glucose, an effect that is more pronounced in patients taking oral hypoglycemic agents plus insulin. This effect was more pronounced in those with lower HbA1c	In T1DM, vaccination can cause a temporary perturbation of interstitial glucose. There is no difference between the AstraZeneca and the Pfizer vaccines	The effects of the first and second vaccination on interstitial glucose regulation could not be compared
Gouda *et al*[57]	TIR, TDD of insulin, dose 1, dose 2, type of vaccine, insulin dosage, average glucose level, bolus insulin, automated bolus	One week after vaccination, there was a slight decrease in TIR along with an increase in mean blood glucose levels, but both were statistically insignificant	No differences in blood glucose or glycemic perturbations were shown before and after vaccination in patients with T1DM. There was no correlation between vaccine side effects and TIR	The effects of the first and second vaccination on interstitial glucose regulation could not be compared

[1]The authors are the same, but the individual studies are different, including different phases, different samples, and different data.

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; BMI: Body mass index; HbA1c: Glycated hemoglobin; TIR: Time in range; TAR: Time above range; TBR: Time below range; CV: Coefficient variation; TDD: Total daily dose; DM: Diabetes mellitus;T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; AESI: Adverse events of special interest; CGM: Continuous glucose monitoring; GMT: Geometric mean titer; GMC: Geometric mean concentration; Abs-RBD-IgG: Anti-SARS-CoV-2 receptor-binding domain IgG; FPG: Fasting plasma glucose; IFG: Impaired fasting glucose; BG: Blood glucose; eGFR: Estimated glomerular filtration rate; COPD: Chronic Obstructive Pulmonary Disease.

here include islet cell injury and acute insulin reduction following entry through the islet angiotensin-converting enzyme 2 (ACE2) receptor[76], cytokine storm[77], oxidative stress, over-activation of the renin-angiotensin-aldosterone system[78], and dysregulation of stress hormone release such as cortisol and catecholamines leading to increased insulin resistance[79]. The vaccine can activate the immune system and inflammatory factors leading to a cytokine storm that reduces pancreatic blood flow or directly impairs β-cell function *via* ACE2 receptors, or the inflammatory response increases the cellular oxidative stress and causes pancreatic fibrosis, resulting in decreased insulin synthesis and secretion and reduced insulin sensitivity in target tissues, thereby elevating blood glucose levels[80]. Pancreatic injury has been reported in individuals following the COVID-19 vaccination, which may be a possible cause of hyperglycemia in individuals following vaccination[81,82]. Of these new-onset diabetic patients listed in Table 2, many exhibited low c-peptide levels, suggesting pancreatic damage. Another possible explanation comes from vaccine excipients, adenoviral vectors, and vaccine SARS-CoV-2 spike protein immunogens that trigger similar mechanisms leading to pancreatic damage and inducing subsequent

hyperglycemic crises. mRNA vaccine was used in 19 of 29 patients and the adenoviral vector vaccine was used in eight. It appears that the mRNA-COVID-19 vaccine was associated with more reports of elevated blood glucose compared to the viral vector vaccine. Although the mRNA-COVID-19 vaccine does not contain an adjuvant, mRNA appears to have self-adjuvant properties that induce autoimmune/inflammatory syndromes and trigger new-onset DM, especially the new-onset T1DM[83].

Vaccination elicits different levels of immune responses within and between individuals and is determined by a range of factors either present within the vaccine, such as the type of adjuvant, or within the host, such as the immune response genes, one or more of which combine to act together. It is important to note that clinicians should remain vigilant for these events, especially for diabetic patients, who require strict glucose monitoring and adequate diabetic treatment in the days following vaccination.

Effect of DM on COVID-19 vaccination

Does vaccination of diabetic patients affect the inherent efficiency of the vaccine? If so, what factors can contribute to these effects?

The efficiency of the vaccine is mainly demonstrated by immunogenicity, neutralizing antibodies, and cellular immunity. Twenty-one of the studies included in this review showed that diabetes decreases the response after vaccination. Marfella *et al*[24] compared the neutralizing antibody titers and antigen-specific CD4 cell responses after the COVID-19 vaccine in a non-diabetic population, a diabetic population with well-regulated glucose (HbA1c ≤ 7%), and a diabetic population with poor regulation (glycosylated hemoglobin > 7%) capacity, the results showed that the rate of neutralizing antibody production and the immune response was significantly reduced in the poorly controlled glycemic population, but that T2DM patients with initially poor glycemic control had improved the immune responses after achieving good glycemic control. Their data underscore the notion that hyperglycemia worsens the immune response and that adequate glycemic control improves the immune response.

The underlying cause of the impaired immune response exhibited by diabetic patients after COVID-19 vaccination is not fully understood and may be related to the dysfunction of the adaptive immune response in diabetic patients. The adaptive immune system can be compromised by poor proliferation in response to antigenic stimuli, impaired production of CD4+ T follicular helper cells, and a reduced ability to produce effector lymphokines. Diabetic patients have reduced numbers of circulating CD4+ cells, reduced CD4+ to CD8+ lymphocyte ratios, reduced lymphocyte proliferative responses, impaired monocytes or macrophages, and defective antigen presentation[84]. Intriguingly, some authors have found that patients with T2DM present with an increased white blood cell counts, but they are more likely to have decreased lymphocytes and more senescent CD4+ and CD8+ T cells[85]. These cells are characterized by overexpression of chemokines (particularly C-X-C motif chemokine receptor type 2) and exhibit altered migratory capacity, resulting in poorer vaccine responses in diabetic patients. In addition, the hyperglycemic environment at the time of vaccination worsens the immunological response and also leads to a decreased immune system response to the vaccine.

Age: Age is one of the most critical factors affecting the production of immunoglobulins and neutralizing antibodies. In general, younger people have a stronger immune response to the COVID-19 vaccine and older people have a reduced immune response to vaccination. B-cell activation is critical for the effectiveness of antibody production, but there are several age-related changes in B-cell function and phenotype. Older adults are usually marked by immune senescence, which may reduce the effectiveness of vaccines[86,87]. The immune response to vaccination is controlled by a delicate balance between effector T cells and follicular T cells, and the aging process disrupts this balance, leading to age-related defects in post-transcriptional regulation, T cell receptor signaling, and metabolic function[88]. The age-related immune responses may be heterogeneous, and co-morbidities and their treatment may also affect the immune response[89]. Therefore, booster vaccines for the elderly may be considered.

Gender: Seven studies observed a stronger immune response after vaccination in women compared to men. Genetic differences as well as sex hormone differences can influence vaccine-induced immunity. X chromosomes express 10 times more genes than Y chromosomes, and differences in gene expression between X and Y chromosomes promote sex differences in vaccine-induced immunity[90]. Testosterone suppresses anti-inflammatory immune cells and promotes a more aggressive T helper cell-type immune response, thereby reducing the immune response to vaccines. In contrast, estrogen has a suppressive effect on pro-inflammatory T cells[91]. In addition, ACE2 receptor expression is influenced by estrogen and correlates with the strength of the immune response[92]. Whether diabetes may interact with gender to influence the extent and persistence of vaccine response is unclear. We found that five of the six studies that observed stronger immune responses in women than in men had study populations from healthcare workers[27,32,35,39,44], and, unquestionably, these studies included a higher proportion of women in their samples, potentially biasing the results.

Type of vaccine and method of vaccination: Surprisingly, Kılınç-Toker *et al*[25] observed that mixed vaccination (CoronaVac plus BioNTech) produced better vaccine efficiency, and similarly, Barocci *et al* [26] found that heterologous vaccination also produced better vaccine efficiency. Wan *et al*[93] observed that two doses of CoronaVac followed by a BNT162b2 heterologous booster may be more effective than

three doses of CoronaVac in a diabetic population. A study comparing the immune responses generated by mRNA-based vaccines and inactivated whole virus particle vaccines found that mRNA-based vaccines induced stronger humoral immune responses and higher levels of cellular responses than inactivated whole virus particle vaccines[94]. Adenoviral vectors carry antigens that can persist for long periods of time. Anti-glycoprotein IgG antibodies persist until day 180 after single-dose vaccination with ChAd3-EBO-Z in phase 1/2a clinics[95], and antibody responses to a single dose of ChAdOx1 (AZD1222) vaccine have a long half-life[96]. The mixed vaccination may combine the respective advantages of the different vaccine types, while the robust humoral response induced by the heterologous booster may be attributed to the extended interval between the primary and booster doses. Extended intervals between booster doses may result in higher neutralizing activity and a more extensive humoral response through germinal center responses, including somatic cell hypermutation and affinity maturation[97]. Evidence from several studies suggests that heterologous inoculation is safe and effective and induces a robust humoral response to SARS-CoV-2, allowing for faster protection of the target population[98-100].

Obesity: Adipose tissue is another metabolic organ with high ACE2 Levels that may exhibit a propensity for SARS-CoV-2 and is also a source of inflammatory adipokines and cytokines that regulate glucose and insulin resistance. A previous study suggested that excess adipose tissue may impede nutrient supply to immune cells[101]. Obesity leads to adipocyte hypertrophy, which induces low levels of inflammation and insulin resistance[102]. In addition, the hyperleptinemia and hyperinsulinemia that accompany the obese state contribute to T-cell dysfunction, leading to impaired immune responses [103]. These mechanisms of immune cell suppression can reduce antibody production after vaccination.

Special Populations: Patients with autoimmune rheumatic diseases, hemodialysis patients, and organ transplant patients, a special group with high comorbidity and impaired immune response, have significantly lower antibody titers established after vaccination, and the persistence of IgG titers may follow different kinetics. Billany *et al*[51] described 94 patients on maintenance hemodialysis (including 43 diabetic patients) at the first dose of vaccine antibody response 28 d after vaccination. The results showed that neutralizing antibodies were detectable in 75 patients (79.8%), and there was no difference in the presence or absence of diabetes on antibody detection in the cohort. Reassuringly, Agur *et al*[104] expressed the same notion. Ajlan *et al*[50] evaluated the efficacy and safety of two different vaccine platforms in 431 patients with liver or kidney solid organ transplants (191 of whom were diabetic patients), and they found no difference in efficacy between the two vaccine platforms in solid organ transplant patients, with response unresponsiveness primarily related to DM. Bieber *et al*[105] also reached similar conclusions. These findings seem to support the notion that both vaccination and booster use in immunodeficient populations are associated with better COVID-19-related outcomes, and therefore, regardless of the presence of diabetes, they should be encouraged to receive booster vaccinations to obtain vaccine protection that may be close to that obtained in the general population after two doses, and that combination or allogenic vaccination is a vaccination strategy worth considering for them.

Adverse reactions: Of the 54 studies included, the earliest study began in November 2020, only two years ago so far. SARS-CoV-2 is a novel virus in the history of human viruses, and the COVID-19 vaccine is even more novel for the human being as a whole, given the incredible speed with which many vaccines were developed during the period of COVID-19. It is too early to observe from just two years how the vaccine affects the life cycle of patients with pre-existing DM, so the effect of the COVID-19 vaccine on the natural course of diabetes is more in the form of observed adverse effects. Ten studies mentioned adverse reactions after vaccination, and only Lee *et al*[32] claimed that diabetes had an increased risk of grade 3 to 4 adverse reactions, while most studies expressed that people with DM were less likely to experience significant side effects after COVID-19 vaccination compared to healthy individuals. The most common systemic side effects are headache, chills, fever, and fatigue, and local effects are pain, redness, and swelling at the injection site. Most side effects are mild and disappear within a few days after vaccination and do not interfere with daily activities. Even for those patients diagnosed with new-onset DM or hyperglycemic crisis, their symptoms resolved rapidly with reasonable treatment, and there was not a single case of death. Although some very rare and serious vaccine-related adverse events have also been reported in myocarditis[106], myocardial infarction[107], and Green-Barre syndrome[21], the vast majority of studies have concluded that vaccination is safe in patients with DM.

Understanding the factors associated with the strength of the immune response to these vaccines and the adverse effects associated with vaccine safety is necessary to optimize vaccination programs. These findings support prioritizing vaccination of vulnerable populations such as diabetes and completing the vaccination cycle, and in countries where conditions permit, promoting the use of booster doses, especially for those special groups with impaired immune responses.

Explanation of "no effect" between DM and vaccination
Of the 54 studies included, a total of 12 studies indicated a "no effect" relationship between DM and

vaccination. Piccini *et al*[53] used two types of vaccines in 39 patients over 16 years of age with T1DM who were vaccinated for the entire cycle and showed that no significant differences were observed in time in range, time in different glucose ranges, mean glucose levels, total daily dose of insulin, or bolus ratios before and after any dose or before and after the entire vaccination cycle. They used a hybrid closed-loop system to exclude the effect on glucose brought about by automatic insulin correction of the treatment system. No serious adverse reactions were reported, although minor post-vaccination side effects were observed. Similarly, another study expressed the same opinion[55]. In a prospective multicenter cohort study analyzing T1DM and T2DM patients as well as healthy controls, it was found that anti-SARS-CoV-2 S receptor binding domain antibody levels after the second vaccination were comparable in healthy controls and in patients with T1DM and T2DM, independent of glycemic control. Papadokostaki *et al*[46] also confirmed this notion. These studies suggest that vaccination has no effect on glycemia in patients with DM, regardless of the vaccine type and before and after vaccination; also, DM has no effect on vaccine efficacy or safety. We analyzed the possible reasons for the differences in the results of these 12 studies compared to other studies: First, when the effect of blood glucose on vaccination was studied, it was done in healthy or special populations and not specifically designed for diabetic populations, for example, Billany *et al*'s study[51] was from a hemodialysis population. In addition, the number of diabetic patients included in these studies was very low. The number of diabetic patients in these two studies was 39 and 35, respectively. Therefore, the results cannot be extrapolated to all diabetic patients. Second, the clinical characteristics of the diabetic subgroups in these studies were not sufficient to explain the heterogeneity of the immune response. The confounding factors of diabetics such as age, type of diabetes, severity of the disease, course of the disease, and therapeutic schedule may affect the results to some extent. Third, heterogeneity in assay methods, differences in the timing of antibody detection (whether it coincides with the lowest value of antibody titers), and differences in the period studied (whether it is affected by a mutant strain that exhibits antibody unresponsiveness) can lead to differences in the immune response to vaccination among vaccinated individuals. Although these differences were faced in other studies as well, it is possible that in these 12 studies, it happened to intersect with more factors and showed inconsistent results with other studies.

Combining the findings of these studies, we can infer that although vaccination gives diabetic patients more possible risk of causing elevated blood glucose than the general population, after vaccination, there is a lower antibody response in diabetic patients compared with healthy subjects, but there is still a considerable amount and intensity of the vaccine immune response, and overall the second dose immune response is higher than the first dose, and diabetic patients with good glycemic control and vaccination with the second dose, the immune response can be significantly improved, and booster vaccination is advocated in special populations subject to immunosuppression, the immune response from mixed vaccination is better than that from a single vaccine type, and heterologous vaccination is better than homologous vaccination.

Advantages and limitations and future directions

This is the first systematic review to date to comprehensively analyze the bidirectional effects of COVID-19 vaccination and DM. First, the question about the interaction of DM and vaccination is a novel one, and our review addresses a very clinically relevant question that both physicians and patients are eager to answer. Second, the studies included in this review include a variety of special populations, including pediatric diabetes, hemodialysis, solid organ transplantation, and autoimmune disease populations, as well as a broad representation of patients with two major types of diabetes, which can inform vaccination strategies for patients with DM on a larger scale. Finally, our study data are from real-world sources, providing real and reliable information for optimizing vaccination in this vulnerable population with DM and providing objective and qualitative evidence for future public policy formulation and optimal vaccine strategies.

Of course, there are some limitations to this systematic review. First, as described in Strengths, the wide representation of the included populations also implies large heterogeneity. Population heterogeneity includes, in addition to the common heterogeneity in demographic characteristics, the health-seeking behavior of these populations and the geographic distribution of the population, and these heterogeneities can introduce bias into the interpretation of the overall results. Second, the small sample size of some studies, with a total of 18 cases (series) reported, and the small proportion of people with DM in some studies limit the ability to test for possible differential effects between subgroups. Third, possibly because of ethical challenges in clinical practice, no randomized controlled studies were found among the included studies, although some authors made their best efforts to reduce potential bias from selection by using PSM methods. Finally, important reports not published in English may have been omitted from this review, or the search strategy failed to capture them.

In the world of the COVID-19 vaccine and DM, many questions remain: How frequent is the new-onset of DM after COVID-19 vaccination? Which component of the vaccine is more likely to cause dysglycemia and will COVID-19 vaccine heterologous vaccination reduce adverse events in patients with diabetes? Our systematic review implies some gaps in the literature that could be addressed in the future. Studies on the effects of COVID-19 vaccination on DM in type 1 and type 2 for comparative analysis and studies on changes in the effects of vaccination on the cellular immunity in patients with

DM and the effects of vaccination on the natural course of pre-existing DM are scarce, and there is a need for longer follow-up or well-designed large-scale studies in the future to further provide an updated and more comprehensive evidence-based basis for the relationship between DM and COVID-19.

CONCLUSION

In conclusion, there is a complex relationship between vaccination and DM with bidirectional effects. Vaccination may contribute to the risk of worsening glycemia in diabetic patients, and diabetic patients may have a lower antibody response after vaccination than the general population, but good glycemic control can significantly improve the immune response.

ARTICLE HIGHLIGHTS

Research background
Both coronavirus disease 2019 (COVID-19) and diabetes pose a serious threat to human health. Vaccination is an effective way to prevent the spread of COVID-19. There are few and conflicting data on the interaction between COVID-19 vaccination and diabetes mellitus.

Research motivation
We searched all current clinical studies to explore the complex relationship between COVID-19 vaccination and diabetes.

Research objectives
We analyzed various factors and possible mechanisms of the interaction between COVID-19 vaccination and diabetes in order to inform the optimal vaccination strategy and clinical management of patients with diabetes.

Research methods
We comprehensively searched PubMed, MEDLINE, and EMBASE online databases and the grey literature of medRxiv and bioRxiv using keywords individually or in combination, with a cut-off date of December 2, 2022. We followed the inclusion and exclusion criteria and studies with quantifiable evidence were included in the full-text review. We also manually searched for important references cited by the included studies.

Research results
A total of 54 studies were included. The earliest study began in November 2020. Thirty studies discussed the effect of diabetes on COVID-19 vaccination, with the majority indicating that diabetes decreases the response to vaccination. Of the other 24 studies on the effect of vaccination on diabetes, most concluded that vaccination was associated with a risk of elevated blood glucose. Twelve of the 54 studies expressed a "no effect" relationship between diabetes and vaccination.

Research conclusions
There is a bidirectional relationship between vaccination and diabetes. Vaccination may contribute to the risk of elevated blood glucose in diabetic patients, and diabetes may have a lower antibody response after vaccination than in the general population, but good glycemic control can significantly improve the immune response.

Research perspectives
Our review reveals a complex relationship between diabetes and vaccination and suggests some gaps in the literature that can be addressed in the future, necessitating well-designed large-scale studies to further provide a more comprehensive basis for the relationship between diabetes and COVID-19.

ACKNOWLEDGEMENTS

FOOTNOTES

Author contributions: He YF designed the study, reviewed literature, and drafted the manuscript; Ouyang J, Hu XD and Wu N retrieved and summarized the literature; Jiang ZG, Bian N and Wang J advised on the review and reviewed the final manuscript; All authors have read and approved the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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Country/Territory of origin: China

ORCID number: Yan-Fei He 0000-0003-4689-5068; Jing Ouyang 0000-0003-2369-8301; Xiao-Dong Hu 0000-0001-8472-6244; Ni Wu 0000-0002-5566-7377; Zhi-Gang Jiang 0000-0003-4539-6988; Ning Bian 0000-0002-9029-1117; Jie Wang 0000-0002-7799-9436.

S-Editor: Li L
L-Editor: A
P-Editor: Fan JR

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SYSTEMATIC REVIEWS

Insights on antioxidant therapeutic strategies in type 2 diabetes mellitus: A narrative review of randomized control trials

Dharmsheel Shrivastav, Pradeep Kumar Dabla, Jitender Sharma, Aroop Viswas, Rashid Mir

Dharmsheel Shrivastav, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur 303002, India

Pradeep Kumar Dabla, Jitender Sharma, Aroop Viswas, Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Delhi 110002, India

Rashid Mir, Department of Medical Lab Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk 71491, Saudi Arabia

Corresponding author: Pradeep Kumar Dabla, MD, Professor, Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Geeta Colony Road, Delhi 110002, India. pradeep_dabla@yahoo.com

Abstract

BACKGROUND
Type 2 diabetes mellitus (T2DM) is a metabolic disease of impaired glucose utilization. Imbalance in generation and elimination of free radicals generate oxidative stress which modulates glucose metabolism and insulin regulation, resulting in the occurrence and progression of diabetes and associated complications. Antioxidant supplements in T2DM can be seen as a potential preventive and effective therapeutic strategy.

AIM
To compare randomized controlled trials (RCTs) in which antioxidants have been shown to have a therapeutic effect in T2DM patients.

METHODS
We systematically searched the electronic database PubMed by keywords. RCTs evaluating the effect of antioxidant therapy on glycaemic control as well as oxidant and antioxidant status as primary outcomes were included. The outcomes considered were: A reduction in blood glucose; changes in oxidative stress and antioxidant markers. Full-length papers of the shortlisted articles were assessed for the eligibility criteria and 17 RCTs were included.

RESULTS
The administration of fixed-dose antioxidants significantly reduces fasting blood sugar and glycated hemoglobin and is associated with decreased malondialdehyde, advanced oxidation protein products, and increased total antioxidant capacity.

CONCLUSION
Antioxidant supplements can be a beneficial approach for the treatment of T2DM.

Key Words: Diabetes; Antioxidants; Oxidative stress; Malondialdehyde; Polyphenols; Antioxidant therapy

Core Tip: Antioxidant supplementation reduces oxidative stress in diabetes. Antioxidant supplementation is a potential therapeutic approach for type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycaemia which arises from resistance or deficiency of insulin secreted from pancreatic beta cells[1]. Obesity and physical inactivity are general well-known risk factors for T2DM as well as its micro (nephropathy and retinopathy) and macrovascular (atherosclerotic cardiovascular disease) complications[2]. According to the World Health Organization, the prevalence and death rate was 470 million and 1.37 million in 2017, respectively, and expected to increase continuously, and the estimated prevalence and death rate in 2025 will be 570.9 million and 1.59 million, respectively[3]. In India, the prevalence of T2DM and impaired fasting glucose was 9.3% and 24.5%, respectively, in 2022. Approximately 45.8% of T2DM patients are aware of their diabetes, 6.1% are taking diabetes medication, and 15.1% have diabetes under control.
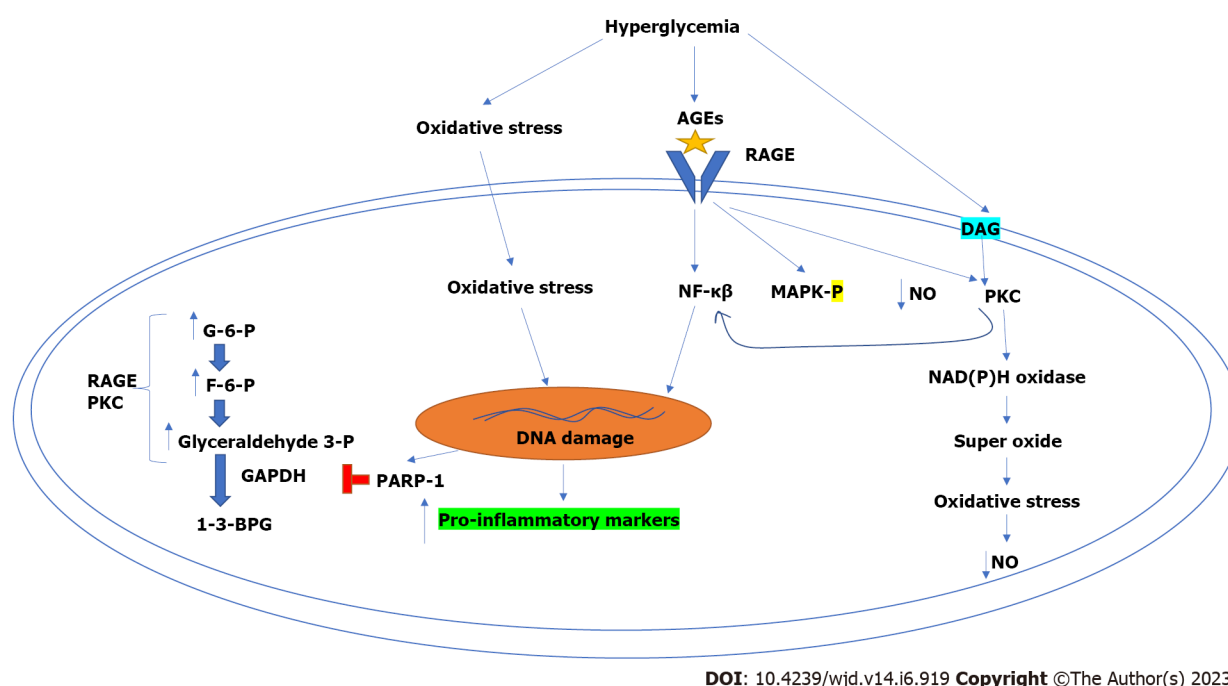
Oxidative stress

Oxidative stress is the excess production or insufficient clearance of highly reactive molecules like reactive oxygen species (ROS) and reactive nitrogen species. In physiological conditions, it is generated in the non-enzymatic, enzymatic, and mitochondrial processes. Enzymes of respiratory chain, phagocytosis, prostaglandin synthesis, and mitochondrial cytochrome P450 system and purine degradation produce free radicals[4]. In diabetes, due to hyperglycaemia, the formation of free radicals is increased, resulting in an increase in oxidative stress which promotes the rate of protein glycation (non-enzymatic), oxidation of glucose, lipid peroxidation, and ultimately impairment of cellular machinery, enzymes, and insulin pathways[5].

Oxidative stress targeted molecular pathways in T2DM pathogenesis

In T2DM, the prolonged exposure to high glucose and free fatty acid levels significantly contributes to the dysfunction of beta cells. These beta cells are highly sensitive to free radicals (due to low quenching and antioxidant activity). Consequently, the oxidative stress can harm mitochondria and significantly decrease insulin secretion and may cause insulin resistance (Figure 1). Under physiological conditions, cellular metabolic processes like glucose oxidation, generate superoxide anion radical [$O_2(-)$] inside the mitochondria which is combated by the antioxidant defence system of the body at a certain level[6]. However, in hyperglycaemic conditions, the production of $O_2(-)$ is elevated, which decreases the body's antioxidant capacity and consequently generates oxidative stress and damage to several biomolecules including DNA[7]. DNA damage activates poly-ADP-ribose polymerase-1 (PARP-1) (DNA damage repair enzyme). Since this PARP-1 enzyme is a potent inhibitor of glyceraldehyde 3-phosphate dehydrogenase of the glycolysis pathway, the intracellular concentration of glycolytic intermediates including glyceraldehyde 3-phosphate, fructose-6-phosphate, and glucose-6-phosphate increases[8]. As a result, glycolytic intermediates accumulate inside the cell and promote some other pro-oxidant pathways like protein kinase C and the advanced glycation end products hexosamine and polyol related pathways[9].

Antioxidants

To counteract the oxidative stress, the human body produce antioxidants at a low concentration which significantly delay or inhibit cellular damage[4]. Humans have extremely complex antioxidant systems that protect the body's cells and organ systems from free radicals. Antioxidants can be categorized as antioxidant enzymes and substrates[10], natural substances[11], combination medications[12], synthetic

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Figure 1 Systematic representation of metabolic pathways affected by hyperglycaemic and oxidative stress. Hyperglycaemia causes cell damage in three ways: (1) By directly increasing oxidative stress which affects glycolytic enzymes; (2) by forming advanced glycation end products which activate NF-κβ and increase DNA damage; and (3) by affecting diacylglycerol enzyme and ultimately reducing nitric oxide levels.

antioxidants[13], and pharmaceuticals[14]. In the antioxidant enzyme and substrate system, superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, and catalase can combat the oxidative stress either directly or sequentially and abolish its excessive development of deleterious effects[15]. The non-enzymatic antioxidant system is endogenously produced and scavenges free radicals. It includes vitamin C, vitamin D, vitamin E, carotenoids, lipoic acid, selenium, and other dietary derivatives such as glutathione and ubiquinol[16].

Antioxidant therapy in diabetes

Exogenous antioxidant supplementation may reduce oxidative stress in T2DM by increasing antioxidant levels and decreasing free radical formation[17]. This supplementation potentially improves the metabolic pathways including nitric oxide (NO) production, endothelial dysfunction, mitochondrial function, and vascular NAD(P)H oxidase activity[18,19]. According to recent clinical data in diabetic patients, supplementation of antioxidants improves glycaemic status [glycated hemoglobin (HbA1c) and random blood sugar], reduces oxidative stress biomarkers [malondialdehyde (MDA)], and increases serum levels of antioxidant enzymes including SOD, catalase, and glutathione peroxidase[5]. Golbidi *et al*[20] investigated the therapeutic use of antioxidants as an adjuvant to standard diabetes treatment. Those authors searched the clinical trial studies over the last ten years using terms vitamin E, vitamin C, coenzyme Q10 (CoQ10), alpha lipoic acid, L-carnitine, ruboxistaurin, or LY 333531 and diabetes and concluded that vitamin supplementation is not beneficial for managing diabetes complications. In this study, we tried to compare interventional randomized control trials (RCTs) in which antioxidants have been shown to have a therapeutic effect in the treatment of T2DM.

MATERIALS AND METHODS

Search methodology: The literature search was carried out in the PubMed NCBI database. The search strategy was carried out by combination of ("Diabetes Mellitus, Type 2"[MeSH]) AND "Antioxidants"[MeSH]) AND "Oxidative Stress"[MeSH]) using Boolean operators. The fixed dose of antioxidant was the inclusion criterion for eligibility.

At the beginning of the literature search, the NCBI PubMed database showed 726 articles. After applying filters and limiting the search with "full text", "five years" (2017 to 2022), and "human randomized controlled trials", 23 RCTs were obtained. Full-length papers of the shortlisted articles were assessed for the eligibility criteria and 17 RCTs that fulfilled the inclusion criteria were finally included in the study (Figure 2 and Table 1).

Table 1 Basic characteristics of included studies

No.	Study design	Setting	Population	Sample size	Intervention	Duration	Effect of treatment	Ref.
1	Randomized controlled trial	Primary Health Care Centre in Podgorica	T2DM patients	Total: *n* = 130; Group I: *n* = 65; Group II: *n* = 65	Group I: 14000 IU vitamin D + metformin; Group II: Metformin only	First for 3 mo and later on for 6 mo	Improves blood HbA1c and reduces advanced oxidation protein products	Cojic *et al*[22], 2021
2	Randomized controlled trial	Prince of Wales Hospital, the Teaching Hospital of The Chinese University of Hong Kong, Shatin, Hong Kong	T2DM patients	Total: *n* = 20; Group I: *n* = 10; Group II: *n* = 10	Group I: 1.4 g/d bilberry (*Vaccinium myrtillus L.*); Group II: Placebo	3 wk	Reduces serum HbA1c level by 4.6% and ascorbic acid by 14%	Chan *et al*[43], 2021
3	Randomized controlled trial	Department of Anesthesia, Isfahan University of Medical Sciences, Isfahan	T2DM patients	Total: *n* = 54; Group I: *n* = 27; Group II: *n* = 27	Group I: Three-gram citrulline daily; Group II: Placebo	2 mo	Reduces serum fasting blood glucose and MDA level by 16% and 25%, respectively; Increases serum levels of NOx, SOD, and GPx by 27%, 2%, and 2.2%, respectively	Azizi *et al*[45], 2021
4	Randomized controlled trial	Khon Kaen University, China	T2DM patients	Total: *n* = 24; Group I: *n* = 12: Group II: *n* = 12	Group I: 1000 mg vitamin C; Group II: Placebo daily	6 wk	Improves blood pressure regulation, increases NO release, and significantly lowers serum MDA and F2-IsoPs levels	Boonthongkaew *et al*[23], 2021
5	Randomized controlled trial	Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, India	T2DM patents	Total: *n* = 60 patients; Group I: *n* = 20; Group II: *n* = 20; Group III: *n* = 20	Group I: One capsule of *T. chebula* 250 mg twice daily; Group II: One capsule of *T. chebula* 500 mg twice daily; Group III: Placebo	12 wk	Improves serum NO level and reduces oxidative stress markers (GSH and MDA)	Pingali *et al*[31], 2020
6	Randomized controlled trial	Endocrinology and Metabolism Clinics of Golestan Hospital at Ahvaz Jundishapur University of Medical Science, Iran (IRCT registration number: IRCT20120704010181N12)	T2DM patients	Total: *n* = 42; Group I: *n* = 21; Group II: *n* = 21	Group I: One-gram *Anethum graveolens* (dill) powder; Group II: Placebo	8 wk	Decreases serum insulin, HOMA-IR, LDL-C, TC, and MDA and increases serum level of HDL and total antioxidant level	Haidari *et al*[33], 2020
7	Randomized controlled trial	Tan Tock Seng Hospital, Singapore (registration number: NCT02776397)	T2DM	Total: *n* = 187; Group I: Type 2 diabetes individuals with haptoglobin 2-2 (Hp 2-2); Group II: Type 2 diabetes individuals without haptoglobin 2-2 (Hp 2-2)	Group I: Total 400 IU of vitamin E daily; Group II: Placebo	24 wk	Increases reactive hyperaemia index, LDL, and ox-LDL concentrations	Dalan *et al*[24], 2020
8	Randomized controlled trial	Isfahan University Endocrine and Metabolism Research Centre, Isfahan, Iran (IRCT registration number: IRCT20180818040827N1	T2DM	Total: *n* = 80; Group I: *n* = 40; Group II: *n* = 40	Group I: 20 g wheat germ; Group II: Placebo	12 wk	Significant change in serum TC level	Mohammadi *et al*[47], 2020
9	Randomized controlled trial	Velayat Hospital of Qazvin University of Medical Sciences, Qazvin, Iran (IRCT registration number: IRCT2017041019669N4)	T2DM	Total: *n* = 62; Group I: *n* = 31; Group II: *n* = 31	Group I: 500 mg of propolis 3 times in a day; Group II: Placebo	8 wk	Decreases FBS, 2-hp, insulin, HbA1c, and HOMA-IR and upregulates TAC, SOD, and GSH	Afsharpour *et al*[40], 2019

10	Double-blind randomized, placebo-controlled clinical trial	Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Kermanshah University of Medical Sciences, Tehran, Iran (IRCT registration number: IRCT20140413017254N5)	T2DM	Total: $n = 80$; Group I: $n = 40$; Group II: $n = 40$	Group I: 80 mg Nano curcumin capsules once a day; Group II: Placebo	8 wk	Improves serum HbA1c, RBS, total neuropathy score, and total reflex score	Asadi *et al*[35], 2019
11	Double-blind, randomized, parallel, placebo-controlled trial	Yeh, Chung Shan Medical University Taiwan (registration number: NCT02622672)	T2DM	Total: $n = 50$; Group I: $n = 25$; Group II: $n = 25$	Group I: Liquid ubiquinol (100 mg/d); Group II: Placebo	12 wk	Reductions in blood HbA1c and fasting glucose, and increase in SOD activity	Yen *et al*[29], 2018
12	Single-blinded randomized controlled clinical trial	Medical Laboratories of the Central Blood Bank Society, and the Medical Relief Society, Gaza Strip, Palestine	T2DM	Total: $n = 40$ patients; Group I: $n = 10$; Group II: $n = 10$; Group III: $n = 10$; Group IV: $n = 10$	Group I: 500 mg of metformin + placebo twice daily; Group II: 500 mg of metformin + 500 mg of vitamin C twice daily; Group III: 500 mg of metformin + 400 mg of vitamin E twice daily; Group IV: 500 mg of metformin + 500 mg of vitamin C + 400 mg of vitamin E twice daily	90 d	Regulates FBS, HbA1c, HOMA-IR, and QISCI and improves GST, MDA, G6PD, GSH-PX, GSHE, and GSHW	El-Aal *et al*[25], 2018
13	Randomized double-blind placebo-controlled trial	Baqiyatallah University of Medical Sciences, Iran (IRCT registration number: IRCT201505301165N4)	T2DM	Total: $n = 100$; Group I: $n = 50$; Group II: $n = 50$	Group I: 500 mg curcumin + 5 mg piperine/day; Group II: Placebo	3 mo	Controls insulin, HbA1c, and HOMA-IR index	Panahi *et al*[36], 2018
14	Randomized, double blind, parallel group design	Clinics Hospital of Porto Alegre	T2DM	Total: $n = 30$; Group I: $n = 15$; Group II: $n = 15$	Group I: n-3 PUFAs (capsules containing 180 mg of eicosapentaenoic acid and 120 mg of docosahexaenoic acid; Group II: Placebo	8 wk	Reduces serum level of TBARS, F2-isoprostanes, and triglycerides	Fayh *et al*[27], 2018
15	Randomized double-blind placebo-controlled trial	Tehran University of Medical Sciences (IRCT registration number: IRCT2015072523336N1)	T2DM	Total: $n = 48$; Group I: $n = 24$; Group II: $n = 24$	Group I: 800 mg/d resveratrol daily; Group II: Placebo	2 mo	Decreases MDA and carbonyl protein and increases total thiol, NOS, and catalase	Seyyedebrahimi *et al*[49], 2018
16	Randomized double-blind placebo-controlled clinical trial	Diabetic Clinic of Golestan Hospital, Jundishapur University of Medical Science, in Ahvaz, Iran (IRCT registration number: IRCT2015081810181N6)	T2DM	Total: $n = 64$; Group I: $n = 32$; Group II: $n = 32$	Group I: 500 mg hesperidin/daily; Group II: Placebo	6 wk	Increases total antioxidant concentration and reduces serum concentrations of fructosamine, 8-OHDG, and MDA	Homayouni *et al*[38], 2017
17	Randomized double-blind placebo-controlled clinical trial	Toho University Medical Center	T2DM	Total: $n = 50$; Group I: $n = 25$; Group II: $n = 25$	Group I: Resveratrol oligo-stilbene 27.97 mg/100 mg/d; Group II: Placebo	12 wk	Decreases SBP and reactive oxygen metabolite significantly and also reduces risk of atherosclerosis in T2DM patients	Imamura *et al*[50], 2017

FBS: Fasting blood sugar; GST: Glutathione S-transferase; G6PD: Glucose 6-phosphate dehydrogenase; GSH: Glutathione; GSHE: Glorisa superba hydroalcoholic extract; HbA1c: Glycated hemoglobin; HDL: High density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; IsoPs: Isoprostanes; LDL: Low-density lipoprotein; LDL-C: Low-density lipoprotein cholesterol; MDA: Malondialdehyde; n-3 PUFA: Polyunsaturated fatty acid; NO: Nitric oxide; NOS: Nitric oxide synthase; 8-OHDG: 8-hydroxy-2'-deoxyguanosine; ox-LDL: Oxidised low-density lipoprotein cholesterol; RBS: Random blood sugar; SBP: Systolic blood pressure; SOD: Superoxide dismutase; T2DM: Type 2 diabetes mellitus; TG: Triglyceride; TBARS: Thiobarbituric acid-reactive substances; TAC: Total antioxidant capacity; TC: Total cholesterol.

RESULTS

This study was performed to find the effect of antioxidants on oxidative stress in T2DM patients by

Figure 2 Flow chart of study selection process. Created from: https://prisma-statement.org/prismastatement/flowdiagram.aspx.

comparing RCT studies. After a literature search in the PubMed database, it was found that the antioxidants, including vitamins, free fatty acids, natural products, *etc.*, play diverse roles in combating oxidative stress in T2DM patients[21]. It is well known that non-enzymatic antioxidants like vitamins A, C, and E, glutathione, lipoic acid, mixed carotenoids, CoQ10, a number of bioflavonoids, antioxidant minerals like copper, zinc, manganese, and selenium, as well as cofactors like albumin, folic acid, uric acid, and vitamins B1, B2, B6, and B12 are involved in diverse biological functions. Antioxidants have shown promise as a potential therapy for the prevention and treatment of cancer, diabetic complications, and cardiovascular disease (CVD) since ROS have been linked to these diseases. In a study by Cojic *et al*[22], vitamin D supplements were given to proven T2DM patients with an average history of 4-6 years during a 6-mo follow-up period, and it was found that vitamin D supplementation (14000 IU weekly or 4 drops daily for 6 mo) improved blood HbA1c and reduced advanced oxidation protein products (AOPP). The triglyceride/thiobarbituric acid-reactive substances (TG/TBARS) index, homeostasis model assessment of insulin resistance (HOMA-IR) index, and MDA level were likewise affected by this vitamin D treatment. Boonthongkaew *et al*[23] studied the effect of vitamin C supplementation (1000 mg daily for 6 wk) on blood pressure (BP), oxidative stress, and NO release in T2DM patients and revealed that vitamin C supplementation improves blood pressure regulation, increases NO release, and significantly lowers serum MDA and F2-isoprostanes (IsoPs) levels. In another study, after supplementation of vitamin E (alpha-tocopherol-400 IU) in T2DM patients (duration of diabetes, 9-11 years), change in the reactive hyperaemia index (RHI) and augmentation index as the primary outcome, and pulse-wave velocity (PWV), carotid intima media thickness (CIMT), inflammation (hsCRP), derivatives of reactive-oxygen metabolites (dROMs), biological antioxidant potential (BAPs), HbA1c, low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and oxidised LDL-C (ox-LDL) as the secondary outcomes were measured. Dalan *et al*[24] concluded that vitamin E supplementation does not significantly improves RHI, PWV, CIMT, hsCRP, dROMS, BAPs, HDL-C, and HbA1c though a significant fall in ox-LDL levels was observed. Further in subgroup analysis, vitamin E supplementation can increase reactive hyperaemia index, LDL, and ox-LDL concentrations in the non-Hp-2-2 group. Similarly, El-Aal *et al*[25] revealed that supplementation of vitamin C and/or vitamins E for 90 consecutive days to T2DM patients regulates fasting blood sugar (FBS), HbA1c, HOMA-IR, and quantitative insulin sensitivity check index (QUICKI). Further, it also improves serum levels of glutathione-S-transferase, MDA, glucose-6-phosphate dehydrogenase, glutathione (GSH)-peroxidase, reduced glutathione in erythrocyte lysate, and reduced glutathione in whole blood. Polyunsaturated fatty acids (*n*-3 PUFAs) are long-chain polyunsaturated fatty acids that have antioxidant properties. Indeed, *n*-3 PUFA supplementation has been demonstrated to reduce oxidative stress-related mitochondrial dysfunction and endothelial cell mortality, with the benefit mediated by increased endogenous antioxidant enzyme activity[26]. In another study conducted by Fayh *et al*[27], supplementation of *n*-3 PUFAs (capsules containing 180 mg of eicosapentaenoic acid and 120 mg of docosahexaenoic acid) to T2DM patients (diabetes history of 6-8 years) non-significantly reduces serum levels of TBARS, F2-IsoPs, and triglycerides. CoQ10 is a powerful antioxidant found naturally in the mitochondria that is endogenously synthesised and fat soluble. Because of its antioxidant properties, it can effectively inhibit the oxidation of fat, protein, and DNA in the body. Deficiency in CoQ10, particularly ubiquinol (the reduced form of CoQ10), is common in T2DM patients[28]. Yen *et al*[29] revealed that supplementing T2DM patients with ubiquinol (100 mg/d for 12 wk) resulted in a significant reduction in blood HbA1c, fasting glucose, and anti-glycaemic agent use (thiazolidinediones by 25% to

83%), and increased SOD activity. However, there were no significant changes in the levels of serum MDA and ox-LDL. After 12 wk of supplementation, there was a further substantial association between the plasma CoQ10 level and the insulin level, HOMA-IR, and anti-hyperglycaemic medication effect scores.

Plant-based natural antioxidants are mostly composed of polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and phenolic acids. These naturally occurring antioxidants, particularly polyphenols and carotenoids, have a variety of biological effects, including anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer properties[30]. *Terminalia chebula*, a traditional ayurvedic herb, is well-known for its antioxidant and antihyperlipidemic properties. Pingali *et al*[31] suggested that the supplementation of aqueous extract of *Terminalia chebula* (250 mg and 500 mg twice daily for 12 wk) to T2DM patients significantly improved endothelial function, serum NO level, lipid profile, hsCRP levels, and oxidative stress markers (GSH and MDA)[31]. Dill, also known as *Anethum graveolens L* (*A. graveolens*), is a herb that is frequently used as a spice and a remedy. The oils of *A. graveolens* are also a source of antioxidants, have antibacterial and antispasmodic qualities, and are also a source of minerals, proteins, and fibres. According to research, *A. graveolens* exhibits anticancer, antibacterial, anti-gastric-irritation, anti-inflammatory, and antioxidant effects[32]. The interventional study of Haidari *et al*[33] suggested that the supplementation of *A. graveolens* (dill) powder (3 capsules per day, 1 g each daily) to T2DM patients (duration of diabetes, 8-9 years) significantly decreases serum insulin, HOMA-IR, LDL-C, total cholesterol (TC), and MDA and increases the serum level of HDL and total antioxidant level. However, a non-significant difference was observed in serum hsCRP (an inflammatory marker) level. Curcumin ($C_{21}H_2OO_6$) is a lipophilic substance and polyphenol in nature. Due to its chemical structure and presence of hydroxyl and methoxy groups, it has many properties, in particular antioxidant, antimicrobial, anti-inflammatory, anti-angiogenic, and antimutagenic ones. Curcumin regulates cyclooxygenase-2, lipoxygenase, xanthine oxidase, and inducible nitric oxide synthase (NOS), and reduces serum level of MDA[34]. In another trial, Asadi *et al*[35] suggested that the supplementation of nano-curcumin (80 mg per day for 8 wk) to T2DM patients (diabetes history of 10-11 years) significantly improves serum HbA1c, random blood sugar, total neuropathy score, and total reflex score. Similarly, the administration of curcuminoids (daily dose of 500 mg/d) co-administered with piperine (5 mg/d for 3 mo) can control insulin, HbA1c, and HOMA-IR index. Further, it also reduces serum hsCRP and creatinine levels in T2DM patients[36]. Hesperidin (30,5,7-trihydroxy-40-methoxy-flavanone-7-rhamnglucoside), a bioflavonoid, is a well-known antioxidant that can reduce risk of cardiovascular disease and T2DM[37]. The oral administration of hesperidin at 500 mg/d for 6 wk in T2DM patients (disease history of 3-11 years) increases total antioxidant concentration (mean percent change 13.35% ± 19.21%) and reduces the serum concentration of fructosamine (mean percent change 10.10% ± 16.84%), 8-hydroxy-2'-deoxyguanosine (mean percent change 25.11% ± 28.23%), and MDA (mean percent change 16.46% ± 18.04%)[38]. Various studies evidently prove that propolis (a resin like material synthesized by honey bee) has antioxidant properties and is sufficiently capable of scavenging free radicals[39]. The oral supplementation of propolis (500 mg, three times a day for 8 wk) to T2DM patients (disease history of 3-11 years) decreases FBS, 2-h postprandial glucose, insulin, HbA1c by 14%, and HOMA-IR by 25%, and upregulates total antioxidant capacity (TAC) by 19%, SOD by 3%, and GSH by 17%[40]. Anthocyanin is one of the major secondary metabolites which have antioxidant properties. Bilberry (*Vaccinium myrtillus L.*) is a natural and big source of anthocyanins[41]. Although bilberry is most typically used to improve vision, it has also been shown to lower blood sugar, have anti-inflammatory and lipid-lowering properties, increase antioxidant defense, and reduce oxidative stress. As a result, bilberry may be useful in the treatment or prevention of inflammation, dyslipidaemia, hyperglycaemia, and elevated oxidative stress, as well as CVD, cancer, diabetes, dementia, and other age-related disorders[42]. The oral supplementation of bilberry (1.4 g/d of extract) daily for 4 wk reduces serum HbA1c level by 4.6% and ascorbic acid by 14%. Further, it decreases serum level of lipid standardized vitamin E, allantoin, glutathione peroxidase, and superoxide dismutase non-significantly[43]. The non-essential α-amino acid L-citrulline plays a major role in liver and kidney regulations. L-citrulline is also beneficial for NO production and endothelial cell regulation[44]. The supplementation of L-citrulline (3 g daily for 2 mo) to T2DM patients (history of 3.5 years) significantly reduces serum fasting blood glucose and MDA levels by 16% and 25%, respectively. However, it significantly increases serum levels of NOx, SOD, and GPx level by 27%, 2% and 2.2%, respectively[45]. Wheat germ (WGEs) is a by-product of the wheat milling process that contains a variety of bioactive chemicals. Wheat germ exracts (WGEs) show potential as antioxidants since they include a variety of bioactive components. According to the findings of a previous study, bioactive compounds present in WGEs lower plasma lipid and oxidation levels[46]. Supplementation of WGEs (20 g per day for 8 wk) to T2DM patients results in a significant change in serum TC level, but it affects neither serum levels of FBS, HbA1C, TG, LDL-C, HDL-C, VLDL, MDA, and TAC, nor HOMA-IR, HOMA-B, QUICKI, TG/HDL ratio, LDL/HDL ratio, systolic blood pressure, and diastolic blood pressure[47].

Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a polyphenolic compound and a type of plant secondary metabolite, is a potent antioxidant which potentially scavenges the free radicals[48]. Oral supplementation of 800 mg/d resveratrol for 2 mo to T2DM patients decreases MDA by 8%, and carbonyl protein by 18.54%. However, it increases total thiol by 12%, NOS by 3%, and catalase 12%.

Further, it also upregulates the expression of nuclear factor erythroid 2-related factor 2 (oxidative stress responsive transcription factor)[49]. Similarly, administration of 100 mg resveratrol tablets (total resveratrol:oligo-stilbene 27.97 mg/100 mg/d) daily for 12 wk effectively regulates arterial stiffness. Resveratrol supplementation not only decreases systolic BP and reactive oxygen metabolite significantly but also reduces risk of atherosclerosis in T2DM patients[50]. In this study, we tried to analyze that how imbalance between the production and inactivation of ROS leads to the development of insulin resistance and metabolic syndrome. Therefore, preventing the damage caused by oxidation can prove to be an effective therapeutic strategy in diabetes. We conducted a comparison of RCTs comparison and performed a review of the available literature to summarize the evidence covering the patho-physiological impact of oxidative stress on type 2 diabetes. Despite these, this study has several limitations including the heterogeneity and lower sample size in RCTs lowering its generalizability. Further, large size randomized controlled trials in populations of different ethnicity and gender are needed to assess its therapeutic implications in T2DM.

DISCUSSION

The literature search revealed that non-enzymatic antioxidants such as vitamins A, C, and E, glutathione, lipoic acid, mixed carotenoids, CoQ10, and antioxidant minerals have diverse biological functions that can potentially prevent and treat cancer, diabetic complications, and cardiovascular diseases. The studies reviewed demonstrated that supplementation of vitamins D, C, and E, *n*-3 PUFAs, and CoQ10 can regulate FBS, HbA1c, and oxidative stress biomarkers such as AOPP, TBARS, and MDA. In particular, vitamin D supplementation significantly improved blood HbA1c and reduced AOPP, while vitamin C supplementation improved blood pressure regulation and significantly lowered serum MDA and F2-IsoPs levels. On the other hand, vitamin E supplementation did not significantly improve RHI, PWV, CIMT, hsCRP, dROMS, BAPs, HDL-C, and HbA1c, but it caused a significant decrease in ox-LDL levels. Furthermore, supplementation of *n*-3 PUFAs non-significantly reduced serum levels of TBARS, F2-IsoPs, and triglycerides, while ubiquinol supplementation resulted in a significant reduction in blood HbA1c, fasting glucose, and anti-glycaemic agent use, and increased SOD activity. However, there were no significant changes in the levels of serum MDA and ox-LDL. These studies highlight the potential benefits of antioxidant supplementation in managing T2DM and the importance of further research to establish optimal dosages, treatment durations, and patient populations.

CONCLUSION

The modern lifestyle, which includes an unhealthy diet, a lack of physical activity, and exposure to a variety of chemicals from various sources such as pesticides, heavy metals, food additives, and environmental pollution, can all influence the appearance of oxidative stress. Oxidative stress plays an important role in the pathogenesis of various metabolic disorders including pre-obesity, obesity, and T2DM. The production of ROS endogenously and/or exogenously is a significant contributor to the development of T2DM and its complications. Constant efforts have been made by researchers globally to develop the therapeutic model to treat T2DM which can ameliorate oxidative stress. In general, oxidative stress can be reduced by adopting a balanced lifestyle and healthy diet. Although nutrition plays a critical role, the supplementation of a diet with antioxidants like vitamins and natural products has the sufficient capacity to downregulate oxidative stress by quenching free radicals and enzymatic and non-enzymatic reactions. It is also suggested that these antioxidants may mitigate T2DM *via* various mechanisms like synchronizing or controlling insulin related cell signalling which can regulate gene replication, transcription, and translation and increase insulin secretion, and improve function of hepatic β cells and glucose reabsorption. Ideally, antioxidant rich food can be taken as part of life in early age. Further, it is also clear that antioxidants are sufficiently capable to reduce low grade inflammation with associated diseases. Also, antioxidant therapy might prove to be beneficial while being supplemented at the late stage of T2DM.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetes mellitus (T2DM) is a condition that affects how the glucose is metabolized for energy. When there is an imbalance between the creation and removal of free radicals, oxidative stress can occur, which affects how the body regulates glucose and insulin, leading to the development and worsening of diabetes and related complications. Taking antioxidant supplements may be a promising way to prevent and treat T2DM.

Research motivation

T2DM is a chronic metabolic disorder with increasing prevalence worldwide, and oxidative stress is implicated in its complications. Antioxidants may counteract this process and can help in improving the metabolic pathways.

Research objectives

To review the current evidence on the role of oxidative stress in the pathogenesis of T2DM and to evaluate the effectiveness of antioxidants as a potential therapy for managing diabetes and its complications.

Research methods

We systematically searched the electronic database PubMed by keywords. Randomized control trials (RCTs) evaluating the effect of antioxidant therapy on glycemic control and oxidant and antioxidant status as primary outcomes were included. The outcomes considered were: A reduction in blood glucose; changes in oxidative stress and antioxidant markers. Full-length papers of the shortlisted articles were assessed for the eligibility criteria and 17 RCTs were included.

Research results

The administration of fixed-dose antioxidants significantly reduced fasting blood sugar and glycated hemoglobin, and was associated with decreased malondialdehyde and advanced oxidation protein products and increased total antioxidant capacity.

Research conclusions

The modern lifestyle and environmental factors can contribute to oxidative stress, which plays a significant role in the development of metabolic disorders such as pre-obesity, obesity, and T2DM. The use of antioxidants through a balanced diet and/or supplementation can reduce oxidative stress, which may mitigate the development and complications of T2DM. Antioxidants can also reduce low-grade inflammation associated with various diseases. Further follow-up research is needed to determine the optimal timing and dosage of antioxidant therapy for diabetic patients.

Research perspectives

Future research should focus on identifying new antioxidants and their mechanisms of action in reducing oxidative stress and preventing or managing T2DM. Additionally, studies on the effectiveness of antioxidant supplementation in combination with other therapies, such as exercise and medication, should be conducted. Further investigation is also needed to determine the optimal timing and dosage of antioxidant supplementation for diabetes prevention and treatment.

FOOTNOTES

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SYSTEMATIC REVIEWS

Usage of topical insulin for the treatment of diabetic keratopathy, including corneal epithelial defects

Ching Yee Leong, Ainal Adlin Naffi, Wan Haslina Wan Abdul Halim, Mae-Lynn Catherine Bastion

Ching Yee Leong, Ainal Adlin Naffi, Wan Haslina Wan Abdul Halim, Mae-Lynn Catherine Bastion, Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur 56000, Wilayah Persekutuan, Malaysia

Corresponding author: Mae-Lynn Catherine Bastion, DO, FRCS, MBBS, Full Professor, Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre, Faculty of Medicine Universiti Kebangsaan Malaysia Jalan Yaacob Latif, Kuala Lumpur 56000, Wilayah Persekutuan, Malaysia. maelynnb2003@yahoo.com

Abstract

BACKGROUND
Diabetic keratopathy (DK) occurs in 46%-64% of patients with diabetes and requires serious attention. In patients with diabetes, the healing of corneal epithelial defects or ulcers takes longer than in patients without diabetes. Insulin is an effective factor in wound healing. The ability of systemic insulin to rapidly heal burn wounds has been reported for nearly a century, but only a few studies have been performed on the effects of topical insulin (TI) on the eye. Treatment with TI is effective in treating DK.

AIM
To review clinical and experimental animal studies providing evidence for the efficacy of TI to heal corneal wounds.

METHODS
National and international databases, including PubMed and Scopus, were searched using relevant keywords, and additional manual searches were conducted to assess the effectiveness of TI application on corneal wound healing. Journal articles published from January 1, 2000 to December 1, 2022 were examined. The relevancy of the identified citations was checked against pred-etermined eligibility standards, and relevant articles were extracted and reviewed.

RESULTS
A total of eight articles were found relevant to be discussed in this review, including four animal studies and four clinical studies. According to the studies conducted, TI is effective for corneal re-epithelialization in patients with diabetes based on corneal wound size and healing rate.

CONCLUSION
Available animal and clinical studies have shown that TI promotes corneal wound

healing by several mechanisms. The use of TI was not associated with adverse effects in any of the published cases. Further studies are needed to enhance our knowledge and understanding of TI in the healing of DK.

Key Words: Diabetes mellitus; Diabetic keratopathy; Topical insulin; Healing

Core Tip: Diabetic keratopathy (DK) is a common complication of diabetes mellitus that is responsible for poor corneal wound healing. It also reduces quality of vision and quality of life. DK is the result of damage resulting from insulin deficiency, hyperglycemia and neuropathy. Topical insulin has been described as an effective and safe new treatment for DK that can normalize the ocular surface and healing rate of epithelial defects. This review examines the available evidence.

INTRODUCTION

Diabetes mellitus is an international public health concern with many complications, both microvascular and macrovascular. The International Diabetes Federation states that 451 million adults worldwide had diabetes in 2017, and this number is expected to increase to 693 million by 2045[1].

Diabetic keratopathy (DK) or diabetic corneal epitheliopathy is one of the complications of diabetes mellitus. It is a degenerative corneal disease that requires serious attention. DK occurs in 46%-64% of patients with diabetes, which affects their quality of life[2]. Ocular surgery, such as corneal transplantation, vitrectomy, and cataract surgery, is a risk factor for corneal epithelial injury in patients with diabetes. Most diabetic keratopathies are thought to occur in the corneal epithelium, but they can also occur in other layers of the cornea, including the corneal stroma, Descemets membrane, and corneal endothelium[3]. DK may present clinically as punctate keratitis, delayed corneal re-epithelialization, corneal hypoesthesia, neurotrophic corneal ulcer and corneal edema. Diabetes increases susceptibility to spontaneous corneal trauma, including epithelial defects and corneal ulcers. In diabetic patients, any corneal epithelial defect or ulcer takes longer to heal and persists longer than in nondiabetic patients[2]. These clinical manifestations are mainly caused by glycation product deposition, corneal nerve ending damage, decreased tear secretion and oxidative stress caused by hyperglycemia. Studies have shown that tear secretion is lower in diabetic patients than in nondiabetic patients[4], and the mucin layer, which forms the innermost layer of tear film, is reduced due to the reduced density of conjunctival goblet cells[5]. In addition, decreased corneal nerve density, which impairs the tear reflex, results in decreased secretion of the aqueous component of tears. Chronic hyperglycemia leads to a decrease in insulin, which plays a role in the proliferation of the acinar cells of the corneal epithelial cells and lacrimal gland[6]. Diabetic microvasculopathy may affect tear secretion by damaging the lacrimal blood supply[7]. Susceptibility, lack of epithelial adherence, decreased corneal nerve plexus and sensitivity are part of the pathogenesis for the development of DK[8].

Insulin is a biologically active peptide closely related to insulin-like growth factor (IGF) that can stimulate the haptotactic migration of human epidermal keratinocytes and is involved in cell growth, proliferation, metabolism and wound healing[9]. The mechanism by which TI improves corneal wound healing is not yet fully understood. Insulin is found in the tear film of the eye. Insulin receptors are found in the corneal epithelium and ocular surface tissue[10]. The presence of insulin and insulin receptors on the cornea and lacrimal glands suggests that insulin may contribute to corneal wound healing[11]. Rocha *et al*[12] also detected insulin in tears and the expression of the insulin receptor and IGF-1 receptor (IGF-1R) on the human ocular surface. IGF-1 promotes corneal epithelial healing by increasing cell proliferation. The topical application of insulin can stimulate IGF-1R and treat DK[12].

MATERIALS AND METHODS

Search strategy

A literature search was conducted and completed on 10 December 2022. Two databases, namely,

PubMed and Scopus, were used to identify all studies concerning topical insulin (TI) treatment for DK. Articles were limited to journal articles with the keywords "topical insulin", diabetes, and keratopathy in the field of the search. The following string was used: TITLE-ABS-KEY ["topical insulin" OR ("local" AND "insulin") OR ("topical" AND "insulin")] AND TITLE-ABS-KEY ("diabetes" OR "diabetic" OR "diabetes mellitus" OR "diabetics") AND TITLE-ABS-KEY ("cornea" OR "corneal" OR "cornea wound healing" OR "corneal wound healing" OR "keratopathy" OR "diabetic keratopathy" OR "cornea wound" OR "corneal wound" OR "eye" OR "eyes"). The search was further supplemented by manual searching for relevant references and using reference citation analysis to find the latest research results. We only examined journal articles published from January 1, 2000 to December 1, 2022.

Inclusion and exclusion criteria

Studies that fulfilled the following criteria were included: (1) The experimental group (adults and animals) was diabetic; (2) The experimental group with DK was treated with TI or insulin-growth-factor; and (3) The effects on the cornea, such as the corneal epithelial defect healing rate, healing size, time to heal, ocular surface disease index score or tear break-up time, were compared between the experimental and control groups. Publications from case reports, letters, and studies without raw data were excluded. We would select either the article with the most recent publication date or with the largest sample size if multiple articles were published based on the same population and were based on one study. In addition, exclusion criteria included articles not published in English, nondiabetic experimental populations, reported effects that were not on the cornea and inadequate information in the article's text.

Data extraction

In the first phase of the search, the first reviewer (Leong CY) reviewed the articles and studies that were duplicated and overlapping were excluded. Subsequently, two reviewers (Leong CY and Naffi AA) independently screened the titles and abstracts, and irrelevant abstract articles were excluded. The full texts of the remaining publications were reviewed by three reviewers (Leong CY, Naffi AA, and Wan Abdul Halim WH), and studies meeting the exclusion criteria were eliminated. Finally, the fourth reviewer (Bastion MLC) reviewed the articles for comprehensiveness and accuracy.

RESULTS

In the first phase of the search, a total of 588 related articles were found with the above strategy. A total of eight articles were found relevant to be discussed in this review, including four animal studies and four clinical studies. The flow chart is presented in Figure 1. All articles in this review are listed in the reference sources. Table 1 contains a list of characteristics of each animal study, and Table 2 illustrates the characteristics of human clinical studies.

DISCUSSION

Animal studies

Nakamura *et al*[13] studied the effects of combining IGF-1 and a substance P-derived tetrapeptide (phenylalanine-glycine-leucine-methionine-amide, or FGLM-NH2) on corneal epithelial wound healing in diabetic rats. The corneal epithelium was removed in both diabetic and nondiabetic rats from limbus to limbus and treated with eye drops containing 1 mmol/L FGLM-NH2 (Peptide Institute, Osaka, Japan) and IGF-1 (1 μg/mL-1) (Becton Dickinson, Bedford, Mass., United States) 6 times daily for 3 d or vehicle alone as a control. The area of the corneal epithelial wound was measured several times for up to 72 h after treatment onset. A delay in wound closure was observed in diabetic rats compared with nondiabetic rats. Similar wound healing processes were observed in normal rats and diabetic rats treated with FGLM-NH2 and IGF-1. However, wound closure was significantly faster in diabetic rats treated with FGLM-NH2 and IGF-1 than in those treated with vehicle[13].

Zagon *et al*[14] performed an animal study and reported that the remaining corneal epithelial defects were 35% larger in rats with diabetes than in healthy animals. In diabetic rats that received TI, corneal healing was significantly enhanced compared to diabetic rats without TI. This study also compared 1, 2, or 5 U insulin in healthy and diabetic rats. Insulin concentrations with more than a 5-fold difference showed no difference in efficacy and safety for the cornea, as determined by corneal thickness, intraocular pressure and ocular surface morphological characteristics[14].

Chen *et al*[15] studied corneal nerve density depletion in patients with diabetes using corneal confocal microscopy and its relationship with TI. The effects of type 1 diabetes on corneal nerves were then studied over time using female Sprague-Dawley rats, whereas the impact of TI on corneal nerves was investigated using female Swiss Webster mice. In rats with diabetes, nerve occupancy in the subbasal plexus was significantly reduced at week 40. TI was applied (0.1 IU daily) to the eyes of diabetes
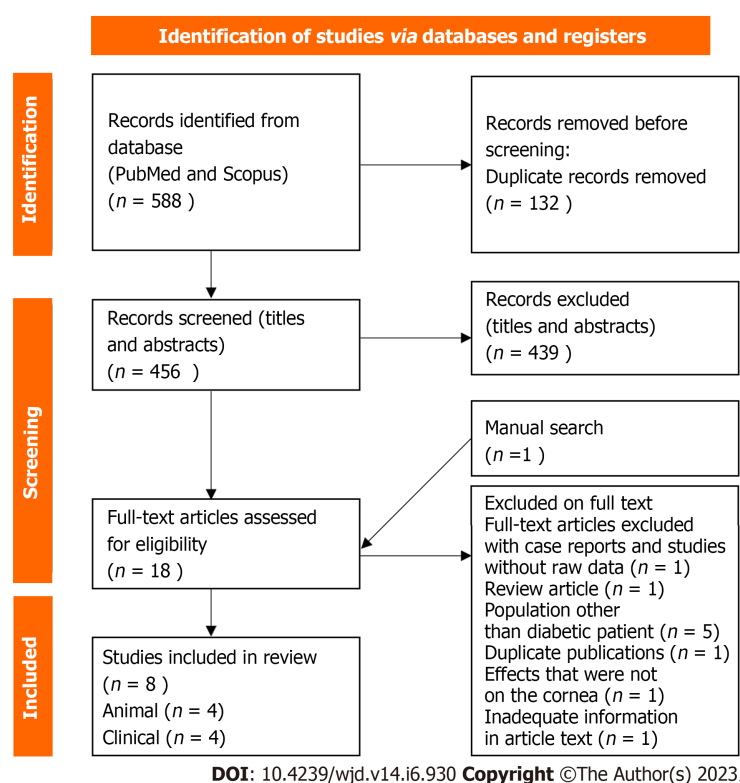
Figure 1 Flow diagram of the literature search.

mellitus rats for 4 wk prevented the depletion of nerves of the subbasal plexus without any effect on systemic glycemic control[15].

Yang *et al*[10] investigated the relationship between TI and the WnT/β-catenin signaling pathway in corneal epithelial healing and corneal nerve repair in diabetic mice. Type 1 diabetes was induced in 6- to 8-year-old male C57BL/6J mice. TI (3 μL) was administered four times daily one week before and one week after corneal scraping. This study showed that TI stimulated the accumulation of β-catenin in the cell, activated the Wnt/β-catenin signaling pathway, and finally stimulated cell proliferation. In addition, a preliminary study of this research showed that TI also promoted epithelial healing in mice with type 2 diabetes after corneal injury[10].

Clinical studies

Bastion and Ling[16] retrospectively reviewed 15 eyes of 14 patients who underwent corneal epithelial debridement during vitreoretinal surgery to improve the surgeon's view in 2010 over a 10-mo period. This study compared three groups: Patients with diabetes treated with TI 1 U/drop four times daily in addition to conventional postoperative therapy, patients with diabetes treated with conventional therapy, namely, topical antibiotics and steroids only, and nondiabetic patients treated with conventional therapy. TI (1 U) was prepared using Actrapid HM, Novo Nordisk, Denmark to provide 50 U/mL insulin at approximately 1 U per drop 4 times per day. Patients with diabetes treated with TI had significantly smaller defect sizes at 24, 36 and 48 h than patients with diabetes treated with conventional therapy. In addition, insulin-treated diabetic eyes re-epithelialized within 48 h, whereas conventionally treated eyes re-epithelialized within 72 h[16].

Fai *et al*[3] prospectively studied the effect of TI at three concentrations (0.5, 1, and 2 U per drop) *vs* placebo four times daily on the postoperative wound healing of corneal epithelium in patients with diabetes after vitreoretinal surgery. This work was a randomized, controlled, double-blind study. Thirty-two eyes of 32 patients with diabetes who underwent intraoperative corneal debridement with a Tookes knife with resulting epithelial defects of various sizes were randomized into 3 different concentrations of TI or placebo. The insulin used was Actrapid HM 100 U/mL, as in the study by Bastion and Ling[16]. The results of this study showed that TI (0.5 U) was superior to the other insulin concentrations in achieving a 100% healing rate within 72 h. TI (0.5 U) 4 times a day (QID) was found to be most effective for healing corneal epithelial defects in patients with diabetes in this study compared to placebo and insulin at higher concentrations after vitrectomy. TI was also shown to be safe for use in the human eye[3].

Aniah Azmi and Bastion[17] evaluated the short-term effects of TI (1 U per drop) four times daily for one month on patients with diabetic dry eye disease (DDED). This work was a randomized, double-blind intervention study involving patients with diabetes with dry eye who were randomly assigned to

Table 1 Summary of the key characteristics of the included animal studies

Ref.	Country	Aim	Study design	Subject groups (number)	Insulin type and dose	Results
Nakamura *et al*[13], 2003	Japan	To study the effect of the combination of FGLM-NH2 and IGF-1 on corneal epithelial wound healing in rats with diabetes	Animal	4-wk-old male Sprague-Dawley Streptozocin-induced diabetic rats; 100 g (*n* = 20)	FGLM-NH2 (1 mmol/L) and IGF-1 (1 µg/mL) 6 times per day	Similar wound healing processes were observed in normal rats and diabetic rats treated with FGLM-NH2 and IGF-1. Wound closure was significantly faster in diabetic rats treated with FGLM-NH2 and IGF-1 than in those treated with vehicle
Zagon *et al* [14], 2007	United States	To determine TI normalizes delayed corneal wound healing in rats with diabetes	Animal	Male Sprague-Dawley Streptozocin-induced diabetic rats; 165 g (38 diabetic rats; 11 nondiabetic rats)	Bovine insulin 1, 2, or 5 U. Single drop (20 µL)	TI normalizes corneal re-epithelialization in diabetic rats. No difference in efficacy of insulin dose of 1, 2, or 5 U and safe for cornea
Chen *et al* [15], 2013	United States	To determine corneal nerve depletion in type 1 diabetes rats using corneal confocal microscopy and its relationship with TI	Animal	Female Swiss Webster Streptozocin-induced diabetic mice; 25-30 g (8 diabetic mice; 8 control)	0.1 IU of regular U-100 Humulin (Lilly, Indianapolis, IN, United States) in 10 µL saline	TI prevent depletion of nerve occupancy in the subbasal nerve plexus of the cornea without affecting systemic glycemic control
Yang *et al* [10], 2020	China	To investigate the relationship between TI and WnT/β-catenin signaling pathway in corneal epithelial healing and corneal nerve repair in diabetic mice	Animal	Streptozocin-induced diabetic mice (6 to 8-year-old-male C57BL/6J mice)	Human neural insulin (Lilly France S.A., Fegersheim, France). 3 µL QID (1 IU/mL)	Insulin contributes to diabetic corneal epithelial wound healing and nerve injury healing *via* Wnt signaling, making it a potential protective factor for diabetic corneal epithelial wounds and nerve injuries

IGF-1: Insulin-like growth factor-1; FGLM-NH2: Substance P-derived tetrapeptide; TI: Topical insulin; U: Unit.

be treated with TI or artificial tears (AT). The insulin used was actrapid HM (Novo Nordisk, Bagsvaerd, Denmark). Patients were assessed at baseline, week 2, and week 4 of treatment. This study showed that TI and AT produced similar improvements in the Ocular Surface Disease Index in the treatment of dry eye in patients with diabetes, whose symptoms had improved after both therapies. However, TI worsened tear break-up time compared with baseline, but this did not differ from that of the AT group. Nevertheless, after one month of treatment, symptoms or clinical signs of DDED did not significantly differ between TI and AT[17].

Quiroz-Mendoza *et al*[18] compared the effect of TI and sodium hyaluronate on the healing of corneal epithelial defects in patients with diabetes after corneal epithelial debridement during pars plana vitrectomy. This study was a controlled clinical trial in which patients were randomly assigned to groups treated with TI 0.5 (IU/drops), 0.15% topical sodium hyaluronate (Hyabak®, Laboratorios Théa® México), or combined treatment with 0.5 IU/drop TI and 0.15% sodium hyaluronate. Insulin was prepared using recombinant human insulin (Humulin® R, Eli Lilly and Company, Indiana, United States). Patients were required to instill TI 4 times per day. Both treatments, *i.e.*, 0.5 IU/drop TI as monotherapy and TI combined with 0.15% sodium hyaluronate, were effective in treating corneal epithelial defects resulting from intraoperative corneal debridement during pars plana vitrectomy in patients with diabetes. The addition of sodium hyaluronate to TI did not provide a greater benefit than TI alone. No adverse effects were noted in this study[18].

Clinical studies on TI in other eye conditions

Wang *et al*[19] reviewed 6 patients with refractory neurotropic ulcers treated with TI. This study was a retrospective study of 6 patients with neurotropic corneal ulcers who did not respond to conventional medical and surgical treatments. The addition of TI resulted in rapid and complete corneal re-epithelialization after the initiation of treatment[19].

Diaz-Valle *et al*[20] evaluated treatment with TI for persistent epithelial defects (PED) refractory to conventional treatment. This study was a prospective, nonrandomized study that enrolled patients with refractory PEDs who did not respond to usual treatment. Patients were treated with insulin eye drops four times daily. This study demonstrated that TI accelerates corneal re-epithelialization and improves and safety promotes healing in PED patients who are not responsive to standard treatment[20].

Tong *et al*[21] reported a case of bilateral neurotropic keratitis that was unresponsive to conventional therapy and was successfully treated with 25 IU/mL TI six times daily in each eye. The neurotropic ulcers dramatically re-epithelialized within 1 wk. In this instance, TI was evidently successful in promoting re-epithelialization where other forms of treatment had failed[21].

Galvis *et al*[22] discussed a case diagnosed with exposure keratopathy after acoustic neuroma resection with involvement of the facial nerve and trigeminal nerve that developed into infectious

Table 2 Summary of the key characteristics of the included clinical studies

Ref.	Country	Aim	Study design	Subject groups (number)	Insulin type and dose	Results
Bastion and Ling [16], 2003	Malaysia	To determine whether TI improve healing rate of corneal epithelial erosion during vitreoretinal surgery	Retrospective review	Human (15 eyes of 14 patients underwent corneal debridement during vitreoretinal surgery)	Actrapid HM, Novo Nordisk 1 U QID (50 UI/mL)	Delayed epithelial healing in diabetic eyes compared with normal eyes. Diabetic eyes treated with TI had significantly smaller defect size than diabetic eyes treated with conventional therapy
Fai *et al*[3], 2017	Malaysia	To investigate the effect of 3 concentration of TI in corneal epithelial wound healing in postoperative patient with diabetes	Double blind randomized controlled	Human (32 eyes of 32 diabetic patient underwent corneal debridement during vitreoretinal surgery)	Actrapid HM, Novonordisk 0.5, 1, 2 U QID	TI 0.5 U QID is most effective for corneal re-epithelialization in patients with diabetes after vitrectomy surgery as compared with placebo and higher concentrations. TI is safe for human ocular use
Aniah Azmi and Bastion [17], 2020	Malaysia	To determine the short-term effects of TI on symptoms and signs of dry eye disease in patients with diabetes	Randomized, double-blind interventional study	Human (320 eyes of 160 patients with diabetes for treatment of dry eyes)	Actrapid HM, Novo Nordisk 1 U QID (25 UL/mL)	Similar improvement in the Ocular Surface Disease Index score for TI 1 U QID and standard artificial tears in the treatment of dry eye in patients with diabetes
Quiroz-Mendoza *et al*[18], 2021	Mexico	To compare the effect of TI and sodium hyaluronate in epithelial defects postoperative in patients with diabetes	Controlled human clinical trial	Human (36 eyes of 36 patients with diabetes who underwent corneal debridement during vitreoretinal surgery)	Recombinant human insulin (Humulin® R, Eli Lilly and Company, Indiana, United States) 0.5 IU/drop QID (25 IU/mL)	TI 0.5 IU/drops monotherapy and combined treatment with 0.15% sodium hyaluronate is effective in healing corneal epithelial defects after intraoperative corneal debridement in patients with diabetes. Adding sodium hyaluronate to TI did not provide additional benefit

TI: Topical insulin; U: Unit; QID: 4 times a day.

keratitis 2 wks after surgery. The patient had a persistent epithelial defect despite topical antibiotics, steroids, autologous serum drops, and bandage contact lenses. TI (1 UI/mL) was administered as adjuvant therapy four times daily, and the epithelial defect closed completely after 2 wks[22].

Ocular surface toxicity from insulin
Bartlett *et al*[23] conducted a prospective, randomized, single-masked study in 8 healthy volunteers on the safety of TI. Subjects were administered different concentrations: 0, 0.1, 1.0, 10.0, and 100 IU/mL TI in one eye and placebo in the other eye. They were evaluated immediately after instillation and 2 h after instillation. Several parameters were measured: Stinging, burning, tearing, itching, foreign body sensation, visual acuity and slit lamp examination. The results showed no significant difference in toxicity between the eyes receiving TI and those receiving placebo[23]. No adverse effect was observed in any of the published cases with the use of TI at concentrations up to 100 IU/mL.

Although all clinical data support the safety of TI, a stable formulation of TI is currently not commercially available. Le Nguyen *et al*[24] first introduced information on the stability of 1 UI/mL insulin eye drops. This study utilized the concentration reported for the effective treatment of refractory epithelial defects in both diabetic and nondiabetic eyes. The physicochemical and microbiological stability of the formulation of TI eye drops were evaluated. TI was prepared by diluting commercial Humalog insulin Lispro solution (100 UI/mL) with polyethylene and propylene glycol-based artificial eye drops to a concentration of 1 UI/mL. The resultant solution was stored in a multidose eyedropper made of low-density polyethylene. The stability of this TI formulation was studied at 4 °C for 12 mo in unopened eyedroppers and under stimulated use conditions at 4 °C and 25 °C for 30 d. The parameters studied for physicochemical stability were visual inspection, pH, turbidity, ultraviolet spectral absorption and osmolality.

In addition, insulin and m-cresol concentrations were tested using a new size-exclusion chromatographic method. The results showed that all tested parameters were favorable, and unopened eye droppers were physicochemically and microbiologically stable at 4 °C for 12 mo. Under stimulated eye conditions, these parameters also remained stable at 4 °C for one month. Furthermore, a similar result was observed when solutions were stored at 25 °C under stimulated eye conditions, with no effect of potential temperature increases on the insulin and m-cresol concentrations in the insulin eyedropper [24]. Studies on the stability of studies utilizing higher concentrations of insulin, such as those described in the various clinical studies on diabetic eyes with epithelial defects or DDED mentioned earlier in this review, are currently lacking. Studies on the stability of TI in various types of AT are also lacking.

Applications of machine-learning analysis

Artificial intelligence (AI) has been developed and used in the field of ophthalmology. The majority of AI research in the past has focused on posterior segment diseases, such glaucoma, retinopathy of prematurity, and optic neuropathy[25]. In recent years, an increasing number of studies have employed AI to recognize different keratopathies. The use of AI in DED, particularly automatic DED detection and categorization, has tremendous potential[26]. The study of DED using machine learning may aid in the diagnosis and monitoring of treatments, such as TI.

Limitations

This study was subject to limitations. The literature included in this study used a variety of types, dosages and methods of dilution of TI. The methodology to assess outcomes in each study, such as cornea wound size and rate, also varied. For animal studies, different types of rats and ages were used, and the sample sizes were small. In addition, the sample sizes of clinical studies were also small, and the types of diabetic keratopathies were different.

CONCLUSION

Treatment with TI is effective in treating DK, including DDED, epithelial defects after corneal debridement and refractory epithelial defects. It offers many advantages, including excellent tolerability, availability, cost-effectiveness and, most importantly, safety when applied to the human eye, without adverse events. More studies are needed to determine its stability in normal saline and in AT of various types, and the advantage of combining TI with AT to increase its contact time and reduce the need for frequent dosing warrants further study.

ARTICLE HIGHLIGHTS

Research background

Diabetic keratopathy (DK) is one of the complications of diabetes mellitus. In diabetic patients, any corneal epithelial defect or ulcer takes longer to heal and persists longer. Treatment with topical insulin (TI) is effective in treating DK.

Research motivation

Insulin is an effective factor in wound healing. The ability of systemic insulin to rapidly heal burn wounds has been reported for nearly a century, but only a few studies have been performed on the effects of TI on the eye.

Research objectives

The aim of the study is to review clinical and experimental animal studies providing evidence for the efficacy of TI to heal corneal wounds.

Research methods

To evaluate the efficacy of TI application on corneal wound healing, the published literature was reviewed systematically for publication. The available data was then thoroughly reviewed.

Research results

Eight articles in total, comprising four animal studies and four clinical studies, were identified and discussed. According to the studies conducted, TI is effective for corneal re-epithelialization in patients with diabetes based on corneal wound size and healing rate.

Research conclusions

Treatment with TI is effective in treating DK. It offers many advantages, including excellent tolerability, availability, cost-effectiveness and, most importantly, safety when applied to the human eye, without adverse events. Further studies are needed to enhance our knowledge and understanding of TI in the healing of DK.

Research perspectives

TI promotes corneal wound healing and was not associated with adverse effects in any of the published cases. More studies are needed to determine its stability in normal saline and in artificial tear (AT) of various types, and the advantage of combining TI with AT to increase its contact time and reduce the need for frequent dosing warrants further study.

FOOTNOTES

Author contributions: Leong CY, Naffi AA, Bastion MLC and Wan Abdul Hamid WH designed the research study; all authors performed the research and screened for relevant articles; Leong CY and Naffi AA analyzed the data and wrote the manuscript; Bastion MLC revised the manuscript and formatted the article; Wan Abdul Hamid WH evaluated the writing and made further amendments to it.

Conflict-of-interest statement: Bastion MLC received fees for serving as a speaker and/or an advisory board member for Novartis, Alcon, and Santen. She received fees for serving as a speaker for Bayer, Lumibird, and Allergan. She has received research funding from Alcon, Novartis, Santen, TRB Chemedica, IDB healthcare, and National University of Malaysia. She is an employee of the National University of Malaysia. Wan Abdul Halim WH received travel funding from Santen. She is also a key opinion leader for Oculus GMBH. She is an employee of Universiti Kebangsaan Malaysia. Leong CY and Naffi AA have no conflict of interest to disclose.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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Country/Territory of origin: Malaysia

ORCID number: Ching Yee Leong 0000-0002-3779-1032; Ainal Adlin Naffi 0000-0002-4682-6620; Wan Haslina Wan Abdul Halim 0000-0001-9140-4040; Mae-Lynn Catherine Bastion 0000-0002-6856-8052.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Cai YX

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