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ABOUT COVER

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The primary aim of *World Journal of Diabetes* (*WJD*, *World J Diabetes*) is to provide scholars and readers from various fields of diabetes with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

   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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Modulatory effect of caffeic acid in alleviating diabetes and associated complications

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Abstract

Diabetes mellitus (DM) is one of the most common metabolic disorders characterized by elevated blood glucose levels. Prolonged uncontrolled hyperglycemia often leads to multi-organ damage including diabetic neuropathy, nephropathy, retinopathy, cardiovascular disorders, and diabetic foot ulcers. Excess production of free radicals causing oxidative stress in tissues is often considered to be the primary cause of onset and progression of DM and associated complications. Natural polyphenols can be used to induce or inhibit the expression of antioxidant enzymes such as glutathione peroxidase, heme oxygenase-1, superoxide dismutase, and catalase that are essential in maintaining redox balance, and ameliorate oxidative stress. Caffeic acid (CA) is a polyphenolderived from hydroxycinnamic acid and possesses numerous physiological properties including antioxidant, anti-inflammatory, anti-atherosclerotic, immune-stimulatory, cardioprotective, antiproliferative, and hepatoprotective activities. CA acts as a regulatory compound affecting numerous biochemical pathways and multiple targets. These include various transcription factors such as nuclear factor-B, tumor necrosis factor-α, interleukin-6, cyclooxygenase-2, and nuclear factor erythroid 2-related factor 2. Therefore, this review summarizes the pharmacological properties, molecular mechanisms, and pharmacokinetic profile of CA in mitigating the adverse effects of DM and associated complications. The bioavailability, drug delivery, and clinical trials of CA have also been discussed.

Key Words: Diabetes mellitus; Caffeic acid; Diabetic foot ulcer; Retinopathy; Nephropathy

Core Tip: Diabetes mellitus has emerged as one of the most common metabolic disorders worldwide which can lead to other complications such as retinopathy, nephropathy, neuropathy, and foot ulcers. Free radical-induced oxidative stress is one of the primary causes of diabetes. Caffeic acid (CA) is a natural polyphenol obtained from various fruits and vegetables. CA and its derivatives act as an antioxidant and regulate the signaling pathways involved in lipid and carbohydrate metabolism. CA also exerts anti-diabetic effects by modulation of inflammatory cytokines and transcription factors.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder marked by elevated blood sugar levels that stems from the complete loss or dysfunction of insulin producing pancreatic β-cells and subsequently results in other complications in several organs of the body.DM is one of the most frequently occurring metabolic diseases worldwide and is the leading cause of death due to comorbidities[1,2]. The main subtypes of DM are type 1 diabetes (T1DM) and type 2 diabetes (T2DM). T1DM, also referred to as insulin dependent DM, is an autoimmune condition that is mediated by the dysfunction of pancreatic β-cells with complete loss of insulin production[3]. T2DM is the insulin resistance type that occurs when pancreatic β-cells are incapable of producing enough insulin. T2DM affects 90%–95% of diabetic individuals globally[4]. Several reports suggest that around 400 million people worldwide would be affected by DM by the year 2025[5]. Both types of DM are frequently linked to long-term consequences such as higher risk of cardiovascular diseases (CVD), retinopathy, neuropathy, nephropathy, foot ulcers, and other vascular anomalies. These complications consequently lead to blindness in diabetic patients, end-stage renal disease, atherosclerosis, and even mortality[6]. Compared to non-diabetic individuals, T2DM patients are at much higher risk of foot injuries and cardiovascular morbidity like atherosclerosis [7]. Studies have demonstrated that metabolic variables, oxidation/glucoxidation, and changes in vascular reactivity are some of the major factors that contribute to diabetic atherosclerosis[8]. Although the pathophysiological mechanism linking DM to its complications is yet to be extensively explored, oxidative stress appears to be a key factor[9-11]. Several reports have suggested that increased oxidative/nitrosative stress and cellular redox disturbances facilitate the etiology and development of both T1DM and T2DM. Uncontrolled hyperglycemia causes oxidative stress and further damages the cells primarily by targeting various metabolic pathways such as enhancement of polyol pathway, increased synthesis of advanced glycation end products (AGEs), activation of protein kinase C, and upregulated hexosamine pathway[9,10]. Therefore, hyperglycemia results in elevated levels of reactive oxygen species and reactive nitrogen species (RNS) in the majority of organs. Moreover, a decrease in cellular antioxidant defences is linked to an increase in oxidative stress in diabetic individuals[9,12,13]. The primary factor contributing to endothelial cell failure in diabetic complications may be due to increased lipid peroxidation caused by oxidative stress. Endothelial dysfunction in DM has been attributed to excessive generation and/or insufficient clearance of free radicals by the antioxidant defencesystem[14] (Figure 1). Since oxidative stress is involved in the development of T1DM, T2DM, and diabetes-associated complications, use of antioxidants as a counter measure could be beneficial. When cells are exposed to chemicals/oxidants, natural polyphenols can be used to induce or inhibit the expression of enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1) that are essential in maintaining cellular homeostasis[15]. Natural polyphenols are secondary metabolites having lower risk of adverse effects when employed in conventional and alternative medicine[16].

Caffeic acid (CA) is a polyphenolic derivative of hydroxycinnamic acid, formed as a product of secondary metabolism in fruits and vegetables[17-19]. CA can be present in simple monomeric form as amides, glycosides, sugar, and organic acid esters, or in complex oligomeric forms as derivatives of flavonoids. CA can also be found attached to some cell wall proteins and polymers[19-20]. CA inhibits the growth of bacteria, fungi, and insects, protects plants from ultraviolet-Bradiations, and contributes to plants' defensive mechanism against predators, pests, and illnesses[21]. Numerous biological effects of CA and its derivatives have been demonstrated through experimental studies, including antibacterial, antiviral, antioxidant, anti-inflammatory, anti-atherosclerotic, immune-stimulatory, cardioprotective, antiproliferative, and hepatoprotective activities[21-25]. Propolis, derived from honeybee, is rich in CA phenethyl ester (CAPE), a common naturally occurring derivative of CA having widespread applications in research and industry[26]. CAPE acts as a regulatory compound affecting numerous biochemical pathways and multiple intracellular targets including several transcription factors, namely,
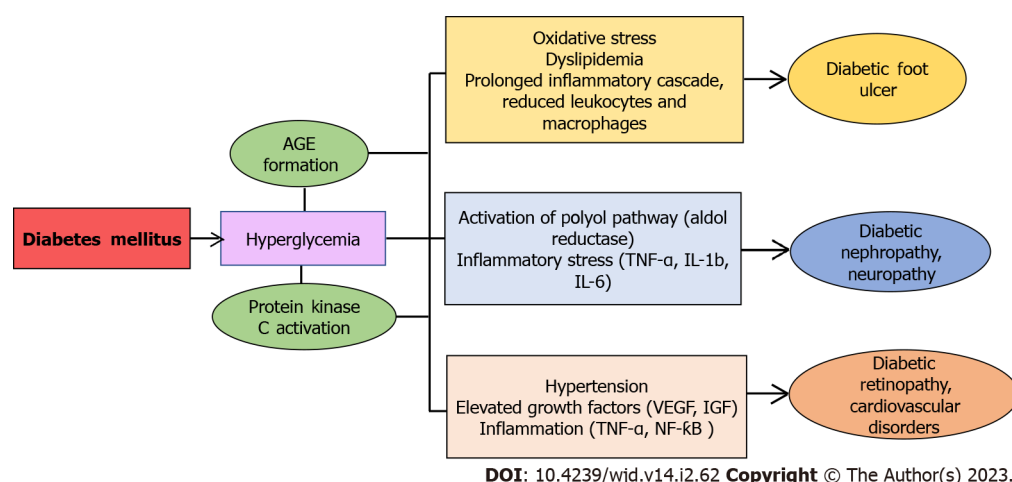
Figure 1 Multiple factors responsible for the onset and progression of diabetes mellitus and associated complications including diabetic nephropathy, neuropathy, retinopathy, and cardiovascular disorders. Hyperglycemia leads to the formation of advanced glycation end products and activation of protein kinase C. This further results in oxidative stress-mediated dyslipidemia, hypertension, activation of polyol pathway, and inflammatory stress. AGE: Advanced glycation end products; TNF-α: Tumor necrosis factor-α; IL: Interleukin; VEGF: Vascular endothelial growth factor; IGF: Insulin-like growth factors; NF-κB: nuclear factor-κB.

nuclear factor-kappa B (NF-κB), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), nuclear factor erythroid 2-related factor 2, inducible nitric oxide synthase (iNOS), activated T-cell nuclear factor, and hypoxia-inducible factor-1[26-30]. Most of these pathways are usually involved in the regulation of inflammatory and oxidative stress markers. Numerous studies have reported the efficacy of CAPE in the treatment of stress-induced pathologies. Recent studies have shown the protective ability of CAPE against nephrotoxicity induced by a number of xenobiotics (methotrexate, doxorubicin, cisplatin, toluene, carbon tetrachloride, *etc.*) or by diverse toxic conditions[31]. Several reports suggest the application of CAPE in experimental and clinical studies for the treatment of several diseases such as cancer, thyroid, liver diseases, hepatic insulin resistance, non-alcoholic fatty liver disease, or hepatocellular carcinoma[32,33]. *In vivo* studies have reported that oral ingestion of CAPE stalled the progression of atherosclerosis in mice deficient in apolipoprotein E[32]. In addition, involvement of CAPE in molecular signaling pathways suggests that CAPE has therapeutic efficacy in diverse inflammatory diseases and cancer[31-32]. Similarly, CA treatment has also exhibited protective efficacy in various organs such as the brain, kidneys, lungs, ovaries, and heart from diabetes-induced damage[34-36]. Therefore, this review reports the structural and pharmacological properties of CA and its derivatives with special emphasis on the key mechanisms of action and pharmacokinetic properties of CA, especially in DM and associated complications.

SOURCES AND CHEMISTRY OF CA

CA occurs naturally in several vegetables and fruits including kiwis, blueberries, plums, cherries, apples, cereals, carrots, and cabbage. CA can also be found in propolis, which is a resinous substance made by honeybees[37]. Different plant species have variable amounts of CA[38]. It is a very prevalent phenolic acid that accounts for 75 to 100 percent of the total hydroxycinnamic acid in fruits[39]. Structurally, CA is a phenylalanine-derived hydroxycinnamic acid with a 3,4-dihydroxyaromatic ring connected to carboxyl group through a trans-ethylene bond[37]. CA is synthesized naturally in plants *via* the endogenous shikimate pathway[23,37]. The biosynthesis of CA begins with precursor shikimic acid and involves three enzymatic reactions: (1) Phosphorylation by shikimate kinase; (2) The conjugation of phosphoenolpyruvate by 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase; and (3) Formation of intermediary metabolite chorismic acid by the enzyme chorismatesynthase[23,37]. Cinnamic acid is produced from the deamination of L-phenylalanine by enzyme phenylalanine ammonia lyase; it is converted into p-coumaric acid by the action of cinnamate-4-hydroxylase, which is subsequently converted into CA by enzyme 4-coumarate 3-hydroxylase[23] (Figure 2). CA is generally extracted from plant materials and by microbial synthesis using organisms like *Escherichia coli*. Two enzymes can be produced by genetic modifications in *Escherichia coli* strains: Tyrosine ammonia lyaseand 3-hydroxylase hydroxyphenylacetate which act on L-tyrosine to produce L-dopa and coumaric acid, respectively, leading to the synthesis of CA[36].
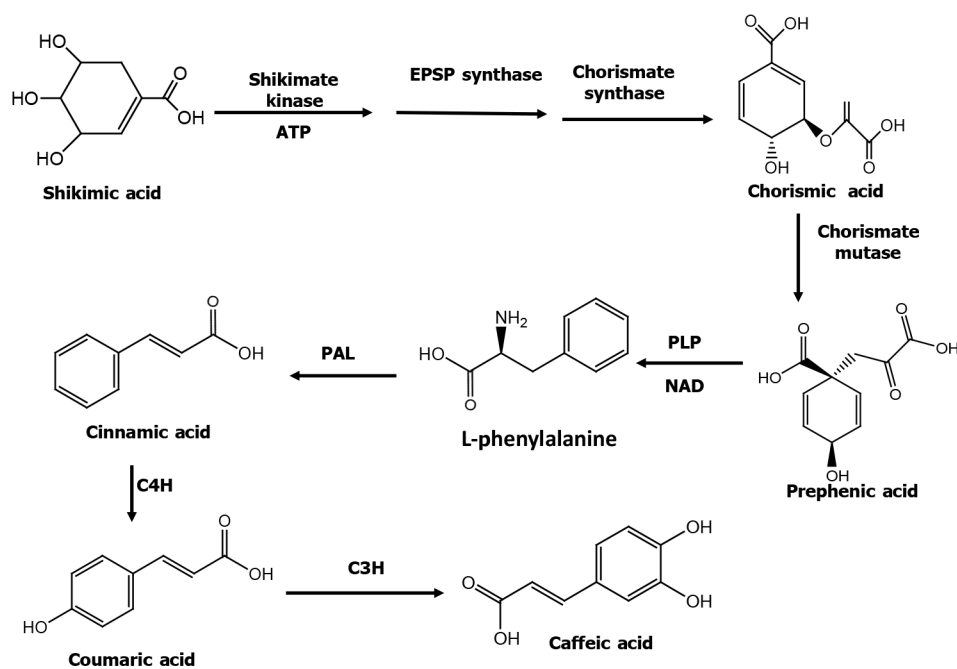
Figure 2 Biosynthesis of caffeic acid *via* the shikimic acid pathway.ATP: Adenosine triphosphate; EPSP: 5-enolpyruvylshikimate-3-phosphate; PLP: Pyridoxal phosphate; NAD: Nicotine adenine dinucleotide; PAL: Phenylalanine ammonia lyase; C4H: Cinnamate-4-hydroxylase; C3H: Coumarate 3-hydroxylase.

PHARMACOKINETICS OF CA

CA has a molecular weight of 180.16 g/mol and is typically found as a white, amorphous powder. The partition coefficient (logP) for CA ranges from 1 to 1.3[40,41]. In addition, propolis contains large amounts of naturally occurring derivative of CA, the CAPE that appears as a white crystalline solid and has a molecular weight of 284.31 g/mol. The intriguing aspect of CAPE is its ability to traverse the blood-brain barrier, which can be attributed to logP values of CAPE ranging between 3.2-13.8[41-43]. CA is essentially found in food in esterified form with chlorogenic acid, thus limiting its absorption in the body[44]. Human tissues such as the intestinal mucosa, stomach, and liver, and biological fluids such as plasma, duodenal fluid, and gastric juice lack the esterase enzymes that hydrolyze chlorogenic acid to release CA. Thus, it is hydrolyzed by intestinal microflora before its absorption[42,44]. As a result, the pharmacokinetic process starts when CA is consumed and enters the stomach in its esterified state, where a small amount of CA is absorbed[41-44]. Thereafter, the intestinal mucosa absorbs up to 95% of CA in its free form after the bacterial esterases in the colon break the ester part of CA[42-44]. Monocarboxylic acid transporters are involved in the active transport of CA across membranes into intestinal cells[36,42,44]. The peak plasma concentration of CA occurs after 1 h of meal digestion, and it takes repeated dosage every 2 h to sustain high levels of CA in plasma[36,42]. Under anaerobic conditions, gut bacteria having tyrosine decarboxylase can cause decarboxylation of CA, producing a compound known as 3-(3-hydroxyphenyl)-propionic acid that has stronger antioxidant activity than CA [45]. Sulfotransferases, uridine diphosphate-glucuronosyltransferases, and catechol-o-methyltransferases catalyze three main enzymatic conjugation processes of sulphation, glucuronidation, and methylation of CA, respectively, that occur immediately after absorption. This increases the hydrophilic properties of CA, thus reducing its toxicity and speeding up elimination. The liver and kidney are the major sites of CA metabolism. The primary elimination route of CA (5.9% to 27%) is *via* urine[43-45].

ANTI-DIABETIC EFFECTS OF CA

DM is characterized by hyperglycemia, altered lipid and carbohydrate metabolism, and oxidative stress [1,2,46]. The successful control of high blood sugar levels with natural polyphenols may be significant in minimizing diabetic complications, particularly micro- and macro-vascular disorders. Plant products used in traditional medicine constitute a potential alternative for effective control of diabetes, owing to their affordability, high efficacy, and minimal negative effects[47-49]. CA is a natural compound that is known to promote insulin secretion, inhibit α-amylase and β-glucosidase, prevent sodium-dependent glucose transporter-1 from absorbing glucose in the gut, and lower hepatic glucose output. Besides its anti-diabetic efficacy, CA also modifies the microbiome, facilitates insulin-dependent glucose uptake,

activates adenosine monophosphate-activated protein kinase, and has immunomodulatory, antimicrobial, hypocholesteremic, and antioxidant properties[36,49]. Experimentally, in streptozotocin (STZ)-induced diabetic rats, and Balb/c and C57BL/KsJ-db/db mice, CA exhibited potential antihyperglycemic effects along with antioxidant and anti-inflammatory properties[50,51]. CA may exert its protective effects by activating and safeguarding intracellular antioxidant enzymes, and by transferring hydrogen atoms and single electrons, as well as by chelating metal ions[52]. In addition, CA helps to upregulate the transcription factor nuclear factor erythroid2-related factor 2 (NrF2) which controls the expression of over 200 genes involved in the cellular antioxidant and immune regulatory mechanism by binding with antioxidant response elements, which is also linked with the detoxification of xenobiotics. CA also regulates β-cell and adipocyte GLUT4 functions, increases activity of glucokinase in hepatocytes, inhibits glucose-6 phosphatase and phosphoenolpyruvate carboxykinase, and reduces glycosylated haemoglobin, thus resulting in controlled DM. CA aids in enhancing the utilization of glucose and glycogen synthases. This leads to reduced cholesterol biosynthesis and prevention of lipogenesis. CA suppresses iron-induced elevation of cholesterol and improves the levels of plasma insulin, C-peptide, and leptin[53]. In a study with STZ-induced diabetic rats, a significant decrease in malondialdehyde (MDA) level and SOD and CAT activities was observed in the liver, retina, and heart, post CA treatment. Insulin-like growth factors (IGFs) are known to be associated with the progression of DM where reduced serum IGF-I levels have been linked with poor glycemic control in DM, while elevated plasma IGF-II levels have been linked to the progression of DM[54,55]. In STZ-induced diabetic rats, the effects of CA administration led to amelioration of changes in gene expression as well as changes in the levels of IGF-I and IGF-II in the blood, liver, heart, and kidney[35,56].

ROLE OF CA IN DIABETES-ASSOCIATED COMPLICATIONS

Diabetic foot and wound healing
Chronic wounds below the ankle or foot lesions in diabetic patients that penetrate the dermis layer are known as diabetic foot ulcers (DFU)[57]. People with diabetes have a lifetime risk of developing foot ulcers in 25% cases, which may lead to 50%–70% of total non-traumatic amputations[58-61]. In recent years, amputation rates have increased significantly, which in turn has raised the rate of morbidity and death[62-65]. The wound healing cascade in diabetic patients is often hindered and delayed due to high blood sugar levels[66]. Hyperglycemia leads to a series of events such as formation of AGEs, non-enzymatic glycosylation, activation of the polyol pathway and the diacylglycerolprotein kinase C pathway, and hyperactivity of the hexosamine pathway[67,68]. These alterations are linked to a prolonged inflammatory phase causing stiffening of endothelial walls, which makes it challenging for blood to pass *via* tiny arteries near the surface of the incision[69]. As a result, there is also a lack of oxygen release and nutrition at the wound site, causing further elevation of blood sugar levels in the wound area. Therefore, the wound healing cascade is prolonged, leukocyte migration is reduced, and macrophage introduction is delayed[70]. Additionally, hyperglycemia also activates an inflammatory reaction by triggering NF-κB light-chain-enhancer of activated B cells[71,72]. Moreover, oxidative stress, dyslipidemia, and insulin resistance play a significant role in the development of DFU[73,74]. Thus, management of all these factors is crucial for the treatment of DFU.

Studies have shown that low glycemic index of CA and its derivatives is mainly responsible for their antidiabetic, antioxidant, and anti-inflammatory properties which aids in managing foot ulcers[75-78]. An early study on STZ-induced diabetic mice revealed that topical administration of propolis is well tolerated and aids in healing of human DFU[79]. CAPE increases wound contraction and re-epithelialization by reducing oxidative stress and accelerates cutaneous wound healing, which is mediated by its antioxidant action[80,81]. In another study on diabetic mice, topical application of propolis was found to stimulate the release of vascular endothelial growth factor (VEGF) in smooth muscle cells and facilitate the relaxation of arteries *via* the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway which accelerated the healing of cutaneous diabetic wounds in mice[82,83] (Figure 3 and Table 1).

Diabetic nephropathy
Diabetic nephropathy is a consequence of prolonged uncontrolled DM causing damage to the renal blood vessel clusters. The pathogenesis of diabetic nephropathy and other complications of diabetes have been linked to non-enzymatic glycation, with the formation of AGEs, also recognized as Maillard reaction products. These AGEs include glycated haemoglobin, glycated albumin, pentosidine, and carboxymethyllysine (CML)[84-86]. In addition, disruption in Th1-Th2 cytokine balance and over-production of pro-inflammatory cytokines result in increased inflammatory stress in diabetic patients, which further accelerates diabetic nephropathy[87,88]. Early investigations have suggested that CA lowers blood glucose by modulating the polyol pathway. Aldose reductase (AR) is the first and rate-limiting enzyme in the polyol pathway that reduces glucose to sorbitol, which could be further metabolised to fructose by the enzyme sorbitol dehydrogenase (SDH)[89,90]. The generation of AGEs was increased by the flux through SDH and an elevated fructose level, which enhanced diabetes-induced microvascular abnormalities[91] (Figure 4). In diabetic mice, CA significantly decreased the

**Table 1 Protective effects of caffeic acid and derivatives in diabetes and associated complications**

Type of study/condition	Dose	Mode of action	Ref.
STZ-induced diabetic mice	Topical administration of propolis at 20 µL	Healing of human DFU	[79]
	CAPE at 5 µmol/kg and 10 µmol/kg	Increased wound contraction and re-epithelialization by reducing oxidative stress	[80,81]
Diabetic mice with DFU	Topical application of propolis	Stimulated VEGF and activated NO/cGMP pathway	[82,83]
Diabetic mice with renal damage	CA at 5%	Decreased AGEs, IL-1b, and IL-6, and reduced activity of renal AR and SDH.	[92]
STZ-induced diabetic mice, nephropathy	CA at 10-50 mg/kg	Modulation of autophagy pathway	[93]
	CA at 40 mg/kg	Improved renal parameters, and downregulated the expression of miR-636	[94]
	CAPE and CAPE-pNO$_2$ at 20 µmol/kg/d	Inhibited inflammation through the Akt/NF-κB pathway and prevented renal fibrosis through the TGF-β/Smad pathway	[95]
Diabetes induced in HUVECs	CAPE treatment at 3-10 µM	Reduced VEGF-induced angiogenesis	[96]
STZ-induced diabetic rats, retinopathy	CAF6 and CAF12 at 250 mM	Modulation of ERK1/2 and protein kinase-B/Akt signaling pathways	[98]
STZ-induced diabetic rats, neuropathy	CAPE at 10 µM/kg/d	Inhibition of iNOS enzyme	[102]
Alloxan-induced diabetic mice, CVD	CA at 50 mg/kg	Reduced atherogenic indices such as TG, LDL-c, VLDL-c, and TC	[53]
	CA at 2%	Improved glycemic control and lipid metabolism, increased plasma antithrombin-III and protein C activities, and decreased MDA, IL-β, IL-6, and TNF-α levels	[34]
STZ-induced T1D rat model, CVD	CAPA at 3 and 15 mg/kg	Reduced myocardial infarction and amelioration of cardiac dysfunction	[103]

STZ: Streptozotocin; DFU: Diabetic foot ulcer; CAPE: Caffeic acid phenethyl ester; VEGF: Vascular endothelial growth factor; NO: Nitric oxide; cGMP: Cyclic guanosine monophosphate; CA: Caffeic acid; AGEs: Advanced glycation end products; IL: Interleukin; AR: Aldol reductase; SDH: Sorbitol dehydrogenase; CAPE-pNO2: Caffeic acid para-nitro phenethyl ester; NF-κB: Nuclear factor-κB; TGF-β: Transforming growth factor-β; HUVECs: Human umbilical vein endothelial cells; ERK: Extracellular signal regulated kinase; iNOS: Inducible nitric oxide synthase; TG: Triacylglycerol; LDL-c: Low density lipoprotein cholesterol; VLDL-c: Very low-density lipoprotein cholesterol; TC: Total cholesterol; T1D: Type1 diabetes; MDA: Malondialdehyde; TNF-α: Tumor necrosis factor-α; CVD: Cardiovascular disorder; CAPA: Caffeic acid phenethyl amide.

production of AGEs, inflammatory cytokines like IL-1b and IL-6, levels of plasma HbA1c, urinary glycated albumin, renal CML, pentosidine, sorbitol, and fructose, and considerably reduced the activity of renal AR and SDH along with suppression of renal *AR* mRNA expression[92]. In an *in vivo* study with STZ-induced diabetic rats, CA in a dose range of 10-50 mg/kg attenuated diabetic nephropathy *via* modulation of autophagy pathway by inhibiting autophagy-regulating miRNAs[93]. In another study on STZ-induced diabetic rats, oral treatment of CA at 40 mg/kg mitigated renal damage and significantly reduced fasting blood glucose, cholesterol, and triglyceride in diabetic rats. CA treatment also improved histological parameters in the diabetic kidney and downregulated the expression of miR-636[94]. In another study in STZ-induced diabetic mice, intraperitoneal treatment with CA derivatives CAPE and CA para-nitro phenethyl ester (CAPE-*p*NO$_2$) at 20 µmol/kg/d resulted in improved renal biochemical parameters such as decreased serum creatinine, MDA, 24-h albumin excretion, blood urea nitrogen, myeloperoxidase levels, and SOD activity in diabetic mice. CAPE and CAPE-*p*NO$_2$ also inhibited inflammation *via* the Akt/NF-κB pathway and prevented nephropathy through the transforming growth factor-β/Smad pathway[95] (Table 1).

Diabetic retinopathy
Long-term DM results in diabetic retinopathy characterized by aberrant retinal blood vessel proliferation and microvascular retinal alterations, resulting in partial vision loss or even complete blindness. One of the major factors causing diabetic retinopathy is VEGF-driven angiogenesis. In a study using human umbilical vein endothelial cells (HUVECs), CAPE treatment in the dose range of 3-10 µM decreased VEGF-induced angiogenesis, indicating possible positive effects in the treatment of diabetic retinopathy[96] (Figure 4). In another study, HUVECs treated with CAPE at 5-20 µg/mL exhibited a
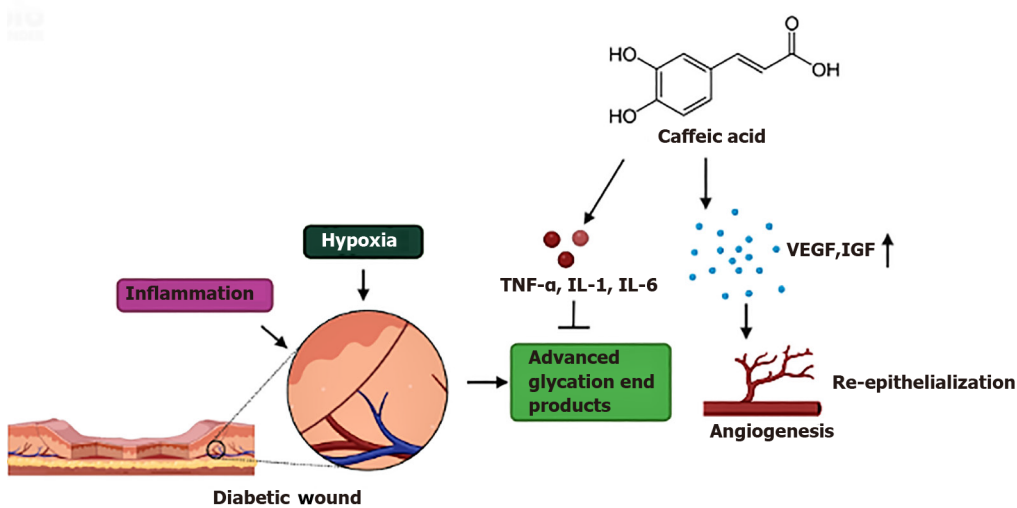
Figure 3 Mechanism of diabetic wound healing mediated by caffeic acid. Hyperglycemia leads to formation of advanced glycation end products (AGEs), hypoxia, and inflammation at the site of injury. Caffeic acid stimulates the inflammatory cascade which inhibits the formation of AGEs and elevates the levels of vascular endothelial growth factorand insulin-like growth factors. This results in vascular angiogenesis and re-epithelialization at the site of injury. VEGF: Vascular endothelial growth factor; IGFs: Insulin-like growth factors; TNF-α: Tumor necrosis factor-α; IL: Interleukin.
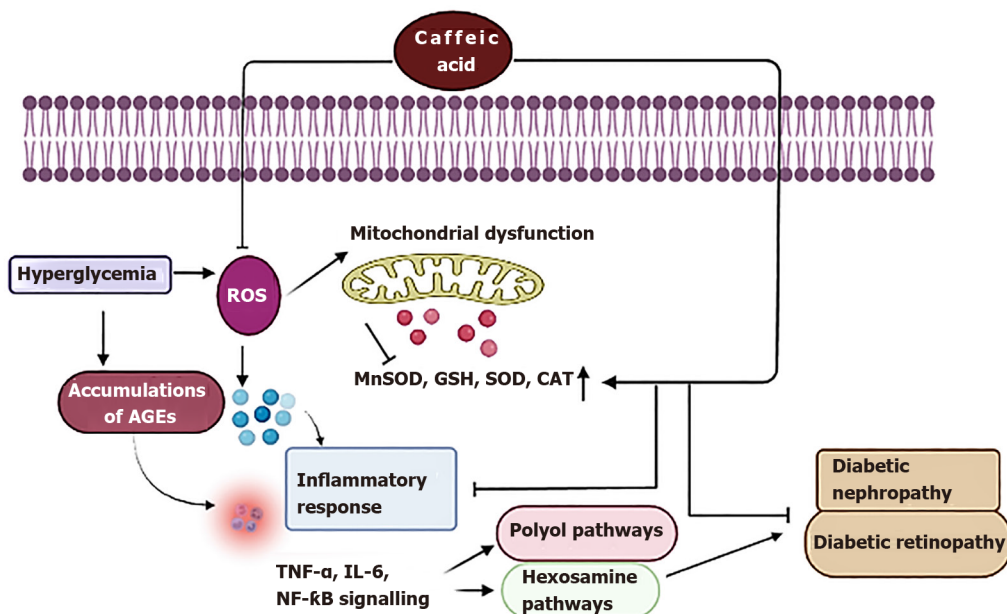
Figure 4 echanism of protective action of caffeic acid in diabetic nephropathy and retinopathy. Hyperglycemia induces formation of advanced glycation end products and reactive oxygen species in renal and retinal tissues, which in turn causes mitochondrial dysfunction by inhibiting antioxidant enzymes such as manganese superoxide dismutase, glutathione peroxidase, catalase, and activates the production of inflammatory cytokines like tumor necrosis factor-α, interleukin-6, nuclear factor-κB, polyol, and hexosamine signaling pathways. Caffeic acid increases the levels of antioxidant enzymes and suppresses inflammatory response, thus protecting the tissues from diabetic nephropathy and retinopathy. AGEs: Advanced glycation end products; ROS: Reactive oxygen species; Mn-SOD: Manganese superoxide dismutase; CAT: Catalase; SOD:Superoxide dismutase; GSH: Glutathione; NF-κB: Nuclear factor-κB; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α.

reduction of VEGF-induced neovascularization and proliferation, tube formation, and migration. The protective efficacy of CAPE can be attributed to the inhibition of VEGF-induced VEGF receptor-2 activation and associated downstream pathways[97]. An *in vivo* study in a STZ-induced diabetic rat model demonstrated the protective efficacy of CA hexyl (CAF6) and dodecyl (CAF12) amide derivatives in diabetic retinopathy. Treatment with CAF6 and CAF12 at a dose of 250 mmol/L led to increased retinal SOD levels, and improved thickness of the whole retinal layer, outer nuclear layer, and ganglion cell count.The CA derivatives ameliorated diabetic retinopathy *via* modulation of the extracellular signal regulated kinase (ERK)1/2 and protein kinase-B/Akt signaling pathways[98] (Table 1).

Diabetic neuropathy and cardiovascular complications

The brain is another organ which is adversely affected by prolonged uncontrolled hyperglycemia, and cerebral dysfunction in diabetic patients is known to be a multifactorial process[99]. Free radical-mediated oxidative stress induced by hyperglycemia plays an important role in the pathogenesis of diabetic neuropathy[100]. It stimulates the production of the inflammatory cytokine TNF-α and promotes the expression of NF-κB[101]. The NO radical in the central nervous system acts as an important regulator leading to the generation of RNS *via* the enzyme iNOS and results in elevated oxidative stress in brain. In an *in vivo* study, STZ-induced diabetic rats post intraperitoneal treatment with CAPE at a dose of 10 μM/kg/day showed reduced NO radical and lipid peroxidation, and increased activities of antioxidant enzymes such as SOD, CAT, and GPx in the rat brain. In addition, CAPE was shown to inhibit the activity of iNOS enzyme, thus preventing excess production of RNS [102].

Hyperglycemia combined with dyslipidemia, oxidative stress, and inflammation cause CVD such as hypertension, cardiac myopathy, and atherosclerosis. DM-mediated CVD is characterized by elevated levels of triacylglycerol (TG), low density lipoprotein (LDL), very low-density lipoprotein (VLDL), and total cholesterol (TC). Atherogenic dyslipidemia in diabetic patients leads to increased risk of cardiac failure. Studies on alloxan-induced diabetic mice have revealed that CA at a dose of 50 mg/kg acts as a potent agent in controlling hyperglycemia and reducing atherogenic indices such as TG, LDL-c, VLDL-c, and TC. Thus, successful restoration of lipid and glucose metabolism parameters in mice by intraperitoneal CA administration led to improved cardiac function[53]. In another study, diabetic mice when orally fed 2% CA, exhibited improved glycemic control and lipid metabolism. CA treatment led to a significant increase in plasma antithrombin-III and protein C activities, and decrease in MDA, IL-β, IL-6, and TNF-α levels[34]. Studies on a STZ-induced T1DM rat model demonstrated that intraperitoneal pre-treatment with CA phenethyl amide at doses 3 and 15 mg/kg led to reduced myocardial infarction and amelioration of cardiac dysfunction[103] (Table 1).

BIOAVAILABILITY AND DRUG DELIVERY OF CA

Plant-derived natural products including CA have several applications in the treatment of a wide range of diseases. However, there are many limitations that come in the way of using phytochemicals as alternative medicine. To ascertain the optimum utilization of plant-derived compounds in clinical investigations, it is important to design novel carriers for the delivery of natural products[104,105]. The use of CA as a pharmaceutical is constrained by a number of physicochemical and pharmacokinetic factors including poor water solubility and lack of specific tissue targeting[106]. Studies have also shown that CA has low oral bioavailability (14.7%) and low intestinal absorption (12.4%) in a rat model [107]. Therefore, numerous nanoparticles (NPs) have been created for the delivery of CA and related compounds in disease therapy with positive outcomes, including polymeric NPs, metal NPs, carbon nanomaterials, and lipid nanostructures[108,109]. The use of NPs for targeted delivery of CA is well reported. The combinations of gold and iron NPs ($Au\text{-}Fe_3O_4$) with CA, quercetin, and 5-fluorocytidine have been formulated for use in breast cancer treatment. Studies related with formulation and development of CA-NPs are mostly targeted for cancer therapy. The release of quercetin and CA from these nanostructures inhibits lactate secretion and prevents glycolytic reprogramming[106,107]. Additionally, NPs have also been designed for CAPE delivery for the treatment of cancer. In a recent study, methoxy poly (ethylene glycol)-b-poly(-caprolactone) was used to create polymeric nanostructures, which were subsequently loaded with CAPE[45,110,111]. There is still much work to be done in terms of NP formulation and design. Hence, further studies are required to examine the potential functions of NPs of CA for better delivery, in treatment of other diseases including DM.

CLINICAL TRIALS AND FUTURE PROSPECTS

Phytometabolites are pharmacologically active compounds and their clinical applications are constantly increasing[45,112,113]. Several reports have shown that approximately one fourth of all clinical compounds used as drugs are derived from natural products[114,115]. The pharmacological action of CA and its derivatives, particularly CAPE, as hepatoprotective, reno-protective, antioxidant, anti-diabetic, anti-inflammatory, and anticancer agents have been well documented. The activity of antioxidant enzymes such as SOD, CAT, and HO-1 is positively modulated by CA. CAPE treatment leads to protection against oxidative stress-mediated diabetic complications by regulating the transcription factors NF-κB, Nrf2, and COX-2 and associated molecular pathways. Moreover, CAPE shows notable efficacy in both *in vitro* and *in vivo* diabetic models with no substantial negative effects. CA exerts anti-diabetic efficacy *in vitro* and *in vivo via* reduced VEGF angiogenesis and decreased MDA, TNF-α, IL-β, IL-6, and other inflammatory and oxidative stress markers.

Table 2 List of clinical trials conducted on propolis against diabetes mellitus

Number	Treatment	Condition	Outcome	ClinicalTrials.gov Identifier, phase, and status of trial
1	Propolis 300 mg twice a day for 12 wk	Type 2 DM	Propolis administration modified the glycemic control in patients with type 2 DM	ClinicalTrials.gov Identifier: NCT03416127 Phase: 2 Status: Completed
2	Propolis 400 mg for 6 mo, after performing scaling and root planing	Type 2 DM, periodontitis	Improvement in HbA1c, FPG, serum CML, and changes in periodontal parameters	ClinicalTrials.gov Identifier: NCT02794506 Phase: 4 Status: Completed
3	Propolis spray at the site of injury	Diabetic foot ulcer	Propolis possesses anti-inflammatory and antioxidant effects and its topical application is well tolerated, improving the healing of human diabetic foot ulcer	ClinicalTrials.gov Identifier: NCT03649243 Phase: Not applicable Status: Completed

DM: Diabetes mellitus, HbA1c: Hemoglobin A1c, FPG: Fasting plasma glucose, CML: Carboxymethyllysine.

In order to examine the clinical trials' data with respect to CA and related compounds in diabetic patients, we searched the largest clinical trial database at 'https://clinicaltrials.gov'. No search results were obtained with the keywords 'CA/CAPE and diabetes'. Since propolis found in beehive is a major source of CAPE, therefore we searched with keywords 'propolis and diabetes' on the database. Three studies were found in which propolis was administered orally or applied topically to diabetic patients. The results are summarized in Table 2.

CA has potential application in the treatment of several diseases including diabetes and associated complications. However, more *in vivo* research needs to be done for a better understanding of the mode of action of CA in DM and associated problems, particularly the role of cytoprotective enzymes like HO-1. Additionally, pharmacokinetic studies are required to entirely understand the metabolic pathway of CA post oral administration. Thus, further clinical investigations in humans are needed to determine the pharmacological potential of CA in major illnesses like diabetes.

CONCLUSION

DM has emerged as one of the most common metabolic disorders worldwide which can lead to other complications such as retinopathy, nephropathy, neuropathy, and foot ulcers. Free radical-induced oxidative stress is one of the primary factors causing DM. CA is a natural polyphenol obtained from various fruits and vegetables. CA and its derivatives act as an antioxidant to regulate the signaling pathways involved in lipid and carbohydrate metabolism. CA also exerts anti-diabetic effects by modulation of inflammatory cytokines and transcription factors. Furthermore, novel delivery strategies are being used for transport of CA to enhance its bioavailability, which has enabled the widespread use of CA in various disease therapies.

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FOOTNOTES

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Insulin: A connection between pancreatic β cells and the hypothalamus

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Abstract

Insulin is a hormone secreted by pancreatic β cells. The concentration of glucose in circulation is proportional to the secretion of insulin by these cells. In target cells, insulin binds to its receptors and activates phosphatidylinositol-3-kinase/protein kinase B, inducing different mechanisms depending on the cell type. In the liver it activates the synthesis of glycogen, in adipose tissue and muscle it allows the capture of glucose, and in the hypothalamus, it regulates thermogenesis and appetite. Defects in insulin function [insulin resistance (IR)] are related to the development of neurodegenerative diseases in obese people. Furthermore, in obesity and diabetes, its role as an anorexigenic hormone in the hypothalamus is diminished during IR. Therefore, hyperphagia prevails, which aggravates hyperglycemia and IR further, becoming a vicious circle in which the patient cannot regulate their need to eat. Uncontrolled calorie intake induces an increase in reactive oxygen species, overcoming cellular antioxidant defenses (oxidative stress). Reactive oxygen species activate stress-sensitive kinases, such as c-Jun N-terminal kinase and p38 mitogen-activated protein kinase, that induce phosphorylation in serine residues in the insulin receptor, which blocks the insulin signaling pathway, continuing the mechanism of IR. The brain and pancreas are organs mainly affected by oxidative stress. The use of drugs that regulate food intake and improve glucose metabolism is the conventional therapy to improve the quality of life of these patients. Currently, the use of antioxidants that regulate oxidative stress has given good results because they reduce oxidative stress and inflammatory processes, and they also have fewer side effects than synthetic drugs.

Key Words: Insulin; Pancreas; Hypothalamus; Hyperphagia; Hyperglycemia; Stress

Core Tip: Insulin is the connection between the β cells of the pancreas and the hypothalamus. Insulin reaches the arcuate nucleus of the hypothalamus and represses the expression of orexigenic neuropeptides to suppress appetite. However, its function decreases when there is damage to the β cells of the pancreas. Its anorexigenic effect decreases and thus increases appetite. The excess of nutrients, specifically carbohydrates, aggravates the damage to β cells and induces obesity and/or diabetes and oxidative damage. The use of antioxidants constitutes a therapeutic approach that has been approached experimentally to regulate the negative effects of alterations in insulin secretion and function.

INTRODUCTION

Insulin is a peptide hormone that plays an important role in glucose homeostasis, cell growth and metabolism[1]. This hormone is synthesized in the β cells of the pancreatic islets; its transcription and translation is regulated in part by nutrients, specifically in response to glucose concentrations[2,3]. The active structure of this hormone is formed by two chains named "chain A" with 21 amino acid residues and "chain B" with 30 amino acid residues linked by three disulfide bonds between both chains[2,4]. The insulin is stored in vesicles to be released into the bloodstream when β cells take up glucose from the extracellular medium[1]; through the bloodstream it will reach all peripheral organs and the brain [5].

Insulin will bind to its receptor in the cell membrane and allow activation of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) insulin signaling pathway[6]. The effects of the activation of this pathway will depend on the cell lineage. It has an anti-atherogenic effect in the vascular system. In the liver, it promotes energy utilization. In the muscle, insulin promotes glucose metabolism and participates in protein synthesis. In adipose tissue, insulin induces lipogenesis[7,8], and finally in the brain it will activate thermogenesis and regulate appetite, glucose homeostasis and metabolism[8-10]. When there are alterations in the secretion or function of insulin, chronic-degenerative pathologies are produced such as hyperphagia, hyperglycemia, insulin resistance (IR) and diabetes mellitus (DM)[11]. A common feature of these pathologies is the formation of reactive oxygen species (ROS), which alter signaling pathways activated by insulin[12-14]. Currently, the use of nutraceuticals has been reported with highly positive effects on the control of ROS and alterations in the secretion and function of insulin at the pancreatic[15] and cerebral levels[16].

INSULIN AND THE PANCREAS

The human pancreas is a retroperitoneal organ in the upper abdomen weighing between 100-150 g and measuring between 15-25 cm in length. It is connected to other abdominal organs such as the spleen, stomach, duodenum and colon[17]. This organ is surrounded by a fibrous capsule that divides its parenchyma into distinct lobes and lobules[18] separated by connective tissue that divides the pancreas into two structurally distinct components: The exocrine pancreas, which consists mainly of acinar cells and duct cells; and the endocrine pancreas, which is the site of islet cells[17,19].

The endocrine portion is composed of groups of cells known as islets of Langerhans, which are attributed with the secretion of several pancreatic peptide hormones for glucose homeostasis, including insulin. There are five major cell types that constitute the islet: α cells; β cells; δ cells; PP cells; and ε cells. They are responsible for producing glucagon, insulin, somatostatin, pancreatic polypeptide and ghrelin, respectively[1,17,20]. The most numerous are the β cells that synthesize and secrete insulin. Insulin is a peptide hormone that was discovered in 1922 by surgeon Frederick Grant Banting and physician Charles Herbert Best and purified by biochemist James Bertam Collip[21,22]. This hormone plays an important role in glucose homeostasis, cell growth and metabolism[1]. In humans, it is encoded by the *INS* gene on chromosome 11, in rats (*Rattus norvegicus*) by the *ins1/2* gene on chromosome 1 and in mice (*Mus musculus*) by the *Ins1* (chromosome 19) and *Ins2* (chromosome 7) genes[23].

The human *INS* gene (1425 bp) is composed of three exons and two introns, as is the rodent *Ins2* gene. However, the rodent *Ins2* gene is composed of only two exons, with the entire coding sequence contained in the second exon[24,25]. In the insulin gene promoter, there are response elements such as

the A element, GG box, C1 [rat insulin promoter element (RIPE)3b1/C2 (RIPE3b2) element (RIPE 3b1/2), cyclic 3′5′-adenosinemonophosphate (cAMP) response element, E element, insulin-linked polymorphic region], enhancer core and Z region where the negative regulatory element is located. These regulatory elements within the promoter region of the insulin gene either enhance or inhibit transcription of the gene and are located between positions -340 and -91 bp relative to the transcription start site.

Several transcription factors bind in these regions including pancreatic-duodenal homeobox-1 protein 1, pair box protein 4 and 6, transcription factor A, hepatocyte nuclear factor-1 alpha and neurogenic differentiation factor 1[2,26,27]. The signal transducer and activator of transcription (STAT) protein also has a very important role in the activation of insulin gene transcription. It has been reported that elevated Ca^{2+} levels activate calpain-1, a protease that cleaves a cytosolic fragment of islet cell autoantigen 512, which promotes transient fusion of the cell membrane with the membrane of insulin-containing granules to release insulin into the extracellular milieu. The free fragment of islet cell autoantigen 512 targets the nucleus and binds to STAT5, which in turn promotes increased transcription of the insulin gene, thus maintaining optimal levels of stored insulin[3].

In addition, there are polypyrimidine tract-binding proteins that positively regulate mRNA translation. Cytosolic polypyrimidine tract-binding protein 1 can bind to pyrimidines, *i.e.* cytosine-uracil-rich sequences in the 3′ untranslated regions of insulin mRNA, thereby stabilizing the insulin mRNA strand and increasing its translation[28].

Insulin translation in pancreatic β cells is regulated in part by nutrients, specifically in response to glucose concentrations[2]. Levels between approximately 2 mmol/L and 4 mmol/L glucose are required to promote insulin biosynthesis and levels greater than 5 mmol/L to promote insulin release[29]. Increased glucose concentrations contribute to the activation of protein phosphatase 1, which dephosphorylates eukaryotic translation initiation factor 2a promoting insulin translation. However, pancreatic endoplasmic reticulum (ER) kinase decreases insulin synthesis through phosphorylation of eukaryotic translation initiation factor 2a[2].

In β cells, insulin is translated as a 110 amino acid pre-proinsulin in the cytosol. Pre-proinsulin contains a 24 amino acid nuclear transport signal peptide (Ala-Ala-Ala-Ala-Pro-Asp-Pro-Gly-Trp-Leu-Ala-Leu-Leu-Leu-Ala-Leu-Leu-Leu-Pro-Leu-Leu-Leu-Arg-Met-Trp-Leu-Ala-Met)[30], which guides pre-proinsulin to the rough ER (RER) membrane for translocation to the RER cisternae *via* two mechanisms: (1) A signal recognition particle (SRP)-dependent cotranslational translocation mechanism where SRP recognizes and binds to the signal peptide of pre-proinsulin arising from ribosomes, forming a complex that interacts with the SRP receptor on the RER membrane, thereby directing nascent pre-proinsulin to the Sec61 translocon[31]; and (2) An SRP-independent post-translational translocation mechanism where in addition to the Sec61 translocon several RER and cytosolic molecular chaperones are involved, including heat shock protein 70, transmembrane recognition complex-4, calmodulin and protein complex Sec 62/63[31].

During translocation, the pre-proinsulin signal peptide must be correctly oriented within the Sec61 translocon so that the N-terminal end of the signal peptide faces the cytosolic side of the RER. This orientation allows the signal peptide cleavage site to be exposed to signal peptidase on the luminal side of the RER membrane[31], generating pro-insulin, a chain of 86 amino acids that folds and stabilizes in its three-dimensional configuration by linking peptide chains A and B through the formation of three disulfide bonds *via* chaperones such as thiol reductase. The first bond is between amino acids CysA6 and CysA11, the second is between amino acids CysA7 and CysB7, and the third bridge is between amino acids CysB19 and CysA20[23,32].

After acquiring three-dimensional folding, pro-insulin is transferred from the RER to the Golgi *via* vesicles where pro-insulin is converted to insulin as these immature vesicles acidify and mature[31] (Figure 1). In the secretory granules there are two endoproteases involved in the conversion of proinsulin to insulin called prohormone convertase 2 (PC2) and PC1/3. The former hydrolyzes between the basic amino acids Arg33-Gly1 at the C-peptide and A-chain junction, and the latter hydrolyzes between the dipeptide Thr30-Arg31 at the B-chain and C-peptide junction[33]. Subsequently, carboxypeptidase E hydrolyzes between the Gln31-Lys32 amino acids as well as between Arg32 and Glu1 basic C-termini of the resulting peptide chains, producing a mature insulin protein of 51 amino acids[23] (Figure 2).

Insulin in its monomeric form tends to form dimers as insulin concentration increases. In the presence of zinc and pH optima (10 mmol/L Zn^{2+}, pH 6.0), the hydrophobic amino acids in the dimeric structures interact and assemble into higher order conformations called hexamers, useful for insulin storage[2]. Once the hexamers are secreted into the circulation by exocytosis, they diffuse into the blood in favor of their concentration gradient. A combination of electrostatic repulsion and decrease in insulin concentration favors the dissociation of insulin into its monomeric form, releasing active insulin and an equimolar proportion of C-peptide[2,33].

This active structure is formed by two chains named "chain A" with 21 amino acid residues and "chain B" with 30 amino acid residues linked by three disulfide bonds between both chains (CysA7-CysB7, CysA20-CysB19 and CysA7-CysA11) (Figure 2). The secondary structure of the A chain contains two antiparallel α-helices connected near the two ends of the A chain. The secondary structure of the B chain contains α-helices and β-strands. This chain can generate two distinct conformations. In a taut
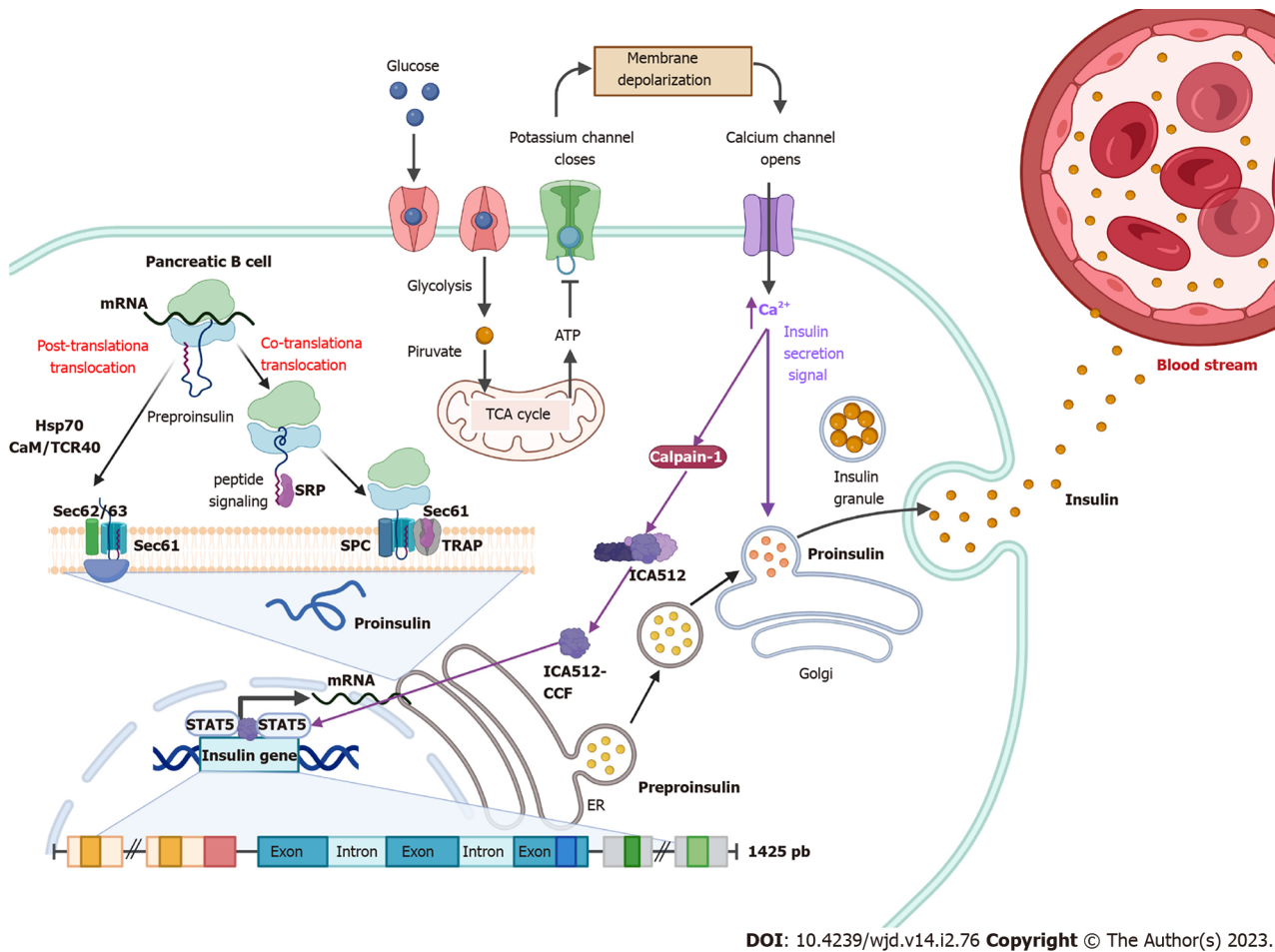
Figure 1 Insulin synthesis. Following food intake, glucose is internalized into pancreatic β cells and its degradation through glycolysis and the tricarboxylic acid cycle is initiated. Intracellular ATP levels increase, which generates the closure of K+ channels, causing a change in membrane permeability opening Ca2+ channels. Elevated Ca2+ intracellular levels activate calpain-1, a protease that cleaves a cytosolic fragment of islet cell autoantigen 512. The free fragment islet cell autoantigen 512 targets the nucleus and binds to signal transducer and activator of transcription 5, which in turn promotes increased transcription of the insulin gene to mRNA. In the cytosol, insulin is translated as pre-proinsulin that includes a nuclear transport signal peptide that guides pre-proinsulin to the rough endoplasmic reticulum (ER) membrane for translocation to the ER cisternae *via* two mechanisms: a signal recognition particle-dependent cotranslational translocation; and a signal recognition particle-independent post-translational translocation mechanism. Pro-insulin is generated and folds and stabilizes in its three-dimensional configuration. After acquiring three-dimensional folding, pro-insulin is transferred from the ER to the Golgi *via* vesicles where pro-insulin is converted to insulin. Also, elevated Ca2+ intracellular levels induce the remodeling of the cytoskeleton and the translocation of insulin granules to the plasma membrane to be subsequently secreted to the blood stream.

state, there is a central α-helix from SerB9 to CysB19 as well as a β-twist from GlyB20-GlyB23 generating a "V" fold. This twist also allows the formation of a β-sheet with Phe24 and Tyr26 in contact with Leu11 and Leu15 of the α-helix of the B-chain. In a resting state, there is a continuous alpha helix from PheB1-CysB19. Disulfide bonds between residues CysA7-CysB7 and CysA20-CysB19 contribute to the stability of the native insulin structure[2,4]. The overall tertiary structure of the protein is highly organized and stabilized by specific interactions involving residues CysA6-CysA11 and LeuA11, PheB1 and LeuB15, IleA2, PheB24, ValA3, IleA13, ValB18 and ValB12 generating a hydrophobic core[2].

Following food intake, glucose is transported into pancreatic β cells *via* the glucose transporter (GLUT) 2 in humans and mice[30,33]. Once pancreatic β cells have internalized glucose and its degradation through glycolysis and the Krebs cycle is initiated, intracellular ATP levels increase, which generates the closure of K+ channels, causing a change in membrane permeability opening Ca2+ channels. This induces the remodeling of the cytoskeleton and the translocation of insulin granules to the plasma membrane to subsequently release the hormone, which through the bloodstream will reach all peripheral organs and the brain[5,30,33] (Figure 1).

Levels between approximately 2 mmol/L and 4 mmol/L glucose are required to promote insulin biosynthesis and levels greater than 5 mmol/L to promote insulin release[29]. Once insulin synthesis is stimulated in the β cells of the pancreas, it is exported through the portal vein to the liver. During this process, more than 50% of the insulin is eliminated by hepatocytes from the liver. The remaining insulin exits through the hepatic vein until it reaches the heart to be distributed through the arterial circulation to the rest of the body to fulfill its various functions. Finally, the remaining circulating insulin is degraded in the kidney[23].
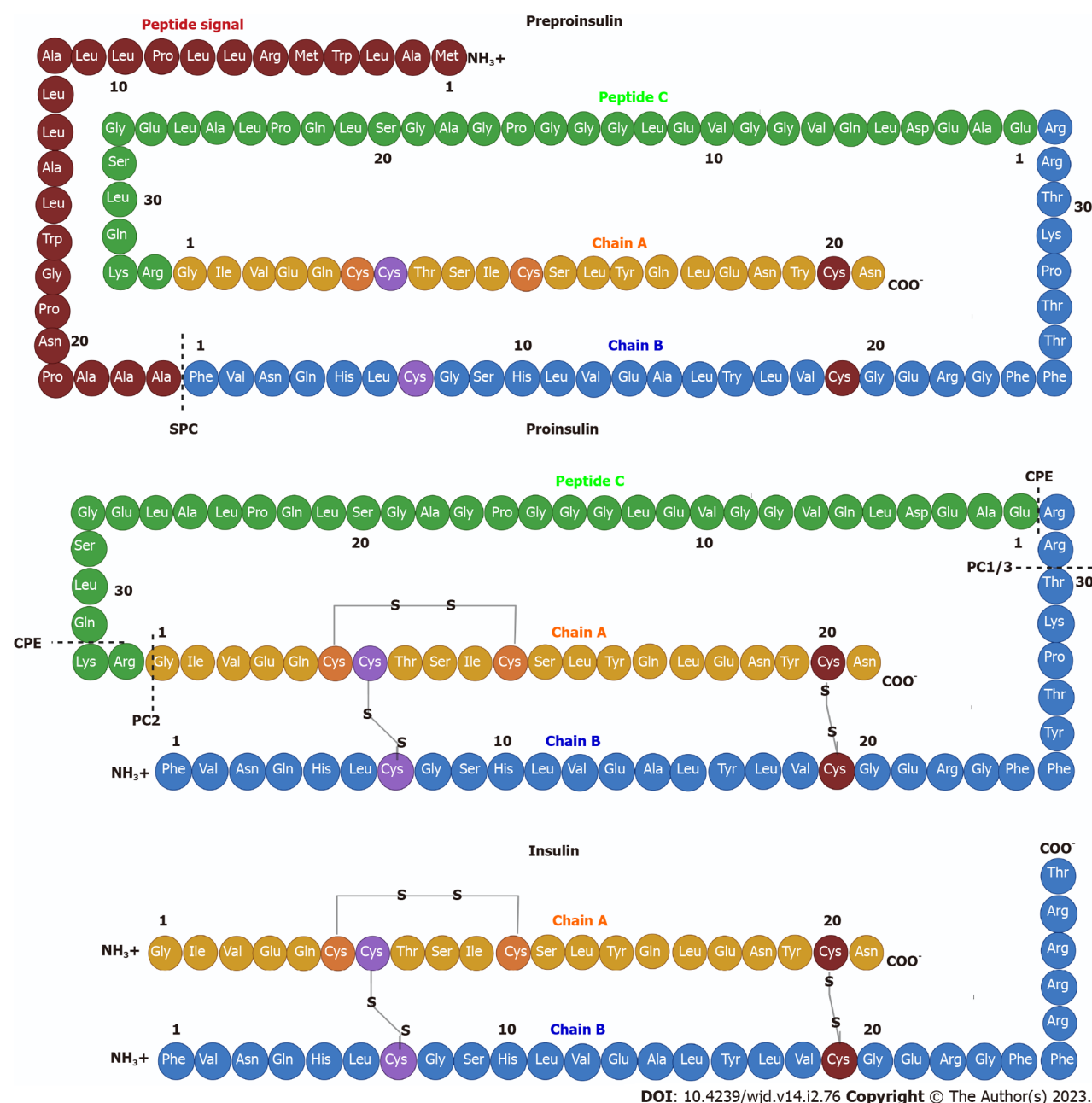
Figure 2 Insulin structure. Pre-proinsulin is secreted as a polypeptide chain of 110 amino acids (aa), composed of a signal peptide (24 aa), chain A (21 aa), peptide C (33 aa) and a chain B (32 aa). The signal peptide is cleaved by signal peptidase generating pro-insulin, a chain of 86 aa that folds and stabilizes in its three-dimensional configuration by three disulfide bonds between both chains: CysA7-CysB7; CysA20-CysB19; and CysA7-CysA11. Finally, two endoproteases, prohormone convertase 2 and prohormone convertase 1/3, hydrolyze between the basic aa Arg33-Gly1 at the C-peptide and A-chain junction and between the dipeptide Thr30-Arg31 at the B-chain and C-peptide junction, respectively. Subsequently, carboxypeptidase E hydrolyzes between the Gln31-Lys32 aa as well as between Arg32 and Glu1 basic C-termini of the resulting peptide chains, producing a mature insulin protein of 51 aa.

In the peripheral organs that depend on insulin to bring glucose into the cells, the hormone will bind to its receptor and allow activation of the PI3K/AKT insulin signaling pathway. This will generate translocation of GLUT4 to the cell membrane thus allowing glucose to enter the cell. Therefore, insulin, through anabolic pathways, regulates blood glucose concentrations[6]. Whereas, the counter-regulatory hormone, glucagon, regulates glucose concentrations through catabolic pathways[1].

Within the positive regulators of insulin, in addition to glucose, are amino acids, glucagon, glucagon-like peptide 1 (GLP-1), growth hormone, secretin, gastrin, glucose-dependent insulinotropic peptide and cholecystokinin. Among the major negative regulators of insulin are adrenocorticosteroids, somatostatin, adrenaline, norepinephrine, neuropeptide Y and calcitonin gene-related peptide[33].

INSULIN SECRETORY DYSFUNCTION

Insulin-secreting β cell dysfunction, defined as the loss of the ability of pancreatic β cells to produce and release insulin in concentrations sufficient to maintain euglycemia, occurs when high and prolonged insulin secretion in response to environmental insults leads to exhaustion of pancreatic β cells[34]. β cells can suffer from insulin secretory dysfunction due to multiple factors. The most common causes are overnutrition (excess nutrients such as glucose and fatty acids), increased body weight, a sedentary lifestyle and aging, which will lead to pathological conditions such as obesity and type 2 DM (T2DM) [34-36]. Other causes of β cell dysfunction, accounting for less than 5% of cases, include diseases that destroy the pancreas, such as acute pancreatitis, chronic pancreatitis and cystic fibrosis[37-39], that specifically inhibit insulin secretion (genetic β cell defects) or that alter counterregulatory hormones (Cushing's syndrome, obesity)[34]. The clinical presentations in these cases depend on the exact nature of the process.

The most common causes of β cell dysfunction share the formation of ROS and cellular oxidative stress as the initiation mechanism[40-42]. Pancreatic β cells are especially vulnerable to stress and oxidative damage[38] due to the low expression of classical antioxidant enzymes such as catalases, glutathione peroxidases and superoxide dismutases compared to other cell types[43,44]. The main antioxidant system of β cells consists of peroxiredoxins, thioredoxins and thioredoxin reductase. This system has been shown to be sufficient to protect β cells against short-term oxidative stress and hypothetically provides a signaling role required for glucose-stimulated insulin secretion in both rodent and human cells[45]. However, long-term glycolipotoxic conditions compromise β cell metabolism and ATP production through glycolytic dysfunction and reduced activation of glyceraldehyde 3-phosphate dehydrogenase, which reduces the generation of pyruvate and promotes β-oxidation.

As a result of metabolic dysfunction, the generation of superoxide and hydrogen peroxide by the mitochondrial electron transport chain is increased[46], increasing cellular ROS concentrations. Excess ROS are capable of oxidizing DNA (mainly mitochondrial DNA), proteins and lipids and function as effector and signaling molecules in cell membranes that mediate signal transduction and inflammation pathways[46,47]. In addition, inflammation, which is also present in the aforementioned pathologies, aggravates the damage and functions as a feedback for stress and oxidative damage because poly-morphonuclear neutrophils at the site of inflammation release large amounts of ROS as an immune defense response, causing tissue damage and endothelial dysfunction[48]. Oxidative stress can induce and maintain a proinflammatory environment through the activation of proinflammatory pathways regulated by the transcription nuclear factor kB and c-Jun N-terminal kinase (JNK) and the production of inflammatory cytokines such as interleukin-1beta[34,38,40,49]. This improves polymorphonuclear neutrophil recruitment, which further stimulates the proinflammatory condition in the tissue, thus generating a feedback process oxidative stress-inflammation-oxidative stress[46].

Persistent inflammation of the pancreas causes ER stress, progressive atrophy and/or replacement with fibrotic tissue, pain, exocrine pancreatic insufficiency, trypsin activation leading to pancreatic autodigestion, loss of functional β cell mass and consequently the reduced ability of β cells to secrete insulin (Figure 3). This pathology is known as pancreatic endocrine dysfunction or DM[50,51].

DM is a complex and heterogeneous disorder defined by the presence of hyperglycemia[11] and can lead to life-threatening complications such as severe hypoglycemia or chronic micro- and macroan-giopathic complications[52]. There are several types of diabetes, although type 1 DM (T1DM) and T2DM are the most common. The American Diabetes Association defines T1DM as the autoimmune destruction of β cells, usually leading to absolute insulin deficiency and T2DM as the progressive loss of insulin action in target tissues as well as a decrease in their secretion from β cells[53]. All cellular events are summarized in Figure 3.

INSULIN AND APPETITE REGULATION

The hypothalamus is the specific area of the brain where eating behavior is regulated, which is directly related to glucose homeostasis[54]. The hypothalamus is located around the third ventricle, below the thalamus and above the median eminence, one of the circumventricular organs in which the blood brain barrier is slightly modified with semi-permeable capillaries that allow selective exchange between molecules of the blood and cerebrospinal flow with the neurons of the hypothalamus[55,56]. This region is divided into several nuclei, among which the arcuate nucleus (ARC), paraventricular nucleus, ventro-medial nucleus, dorsomedial nucleus and lateral area nucleus stand out[57,58]. The ARC is located very close to the median eminence. It is made up of first-order neurons that first receive signals from peripheral organs such as the stomach, adipose tissue and the pancreas[56,59].

Insulin is the signal derived from the pancreas in response to the presence of nutrients (glucose) in the bloodstream[54]. After being secreted from pancreatic β cells, insulin *via* the bloodstream reaches the hypothalamus crossing the median eminence or crossing the vascular endothelium *via* transport proteins or *via* the insulin receptor itself, which is assumed to also act as its transporter (mechanism not fully defined)[60,61].
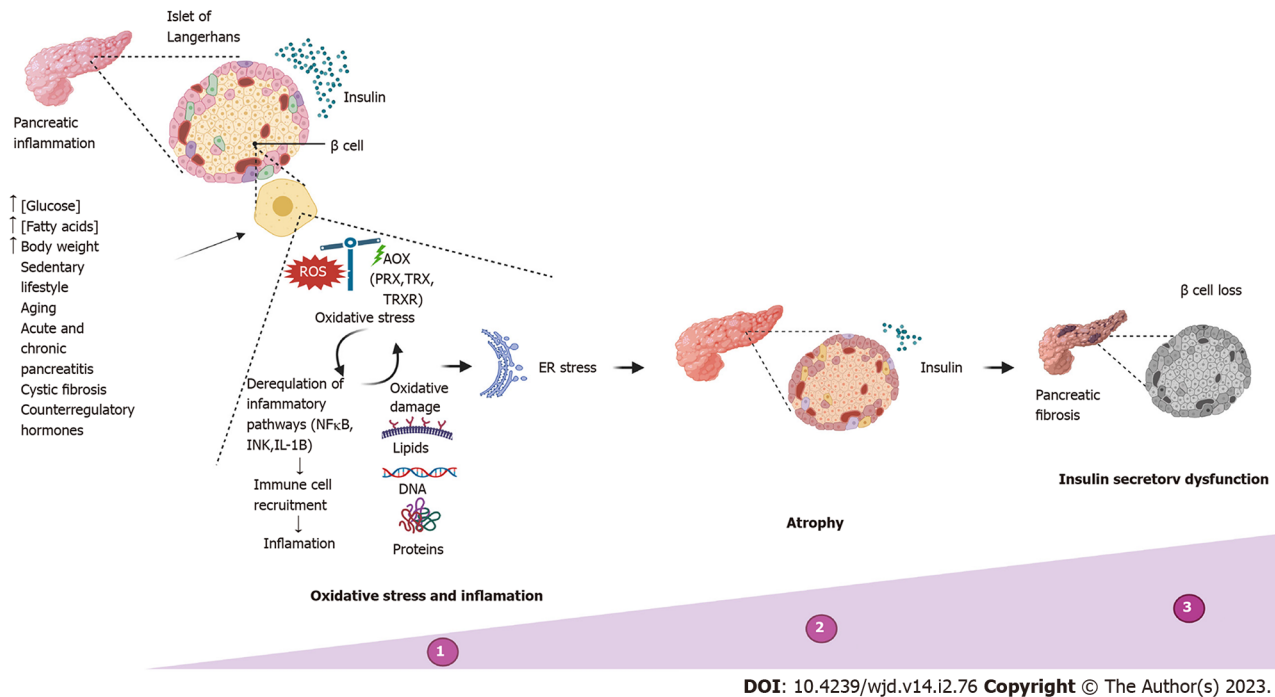
Figure 3 Insulin secretory dysfunction. Timeline of abnormalities in insulin secretion due to the most common causes, reflecting progressive deterioration in functional β cell mass. ER: Endoplasmic reticulum.

Insulin reaches the ARC and binds to its receptor in the first-order neurons. Once insulin binds to its receptor in the hypothalamus, it leads to rapid autophosphorylation of the insulin receptor, followed by tyrosine phosphorylation of insulin receptor substrates, which induces the activation of the PI3K/AKT and the mitogen-activated protein kinases (MAPK) cascades[61]. The PI3K/AKT pathway promotes the activation of the mammalian target of rapamycin complex 1/p70-S6 kinase[61,62], which is capable of phosphorylating AMP-activated protein kinase (AMPK) at serine 485/491 sites[63], reducing the ability of Ca2+/calmodulin-dependent kinase II to phosphorylate AMPK in the threonine 172 residue and resulting in the low expression of genes related to appetite induction (orexigenic), such as neuropeptide Y (*NPY*) and agouti-related protein (*AgRP*) in the ARC, the paraventricular nucleus and the lateral area nucleus, which decreases appetite[56,63,64].

Moreover, AKT induces the phosphorylation of the transcription factor forkhead box protein O1. When forkhead box protein O1 is phosphorylated it leaves the nucleus and therefore decreases the expression of genes that are activated by this factor, such as *NPY* and *AgRP*[56,64]. Therefore, insulin and the activation of it signaling pathway promotes an anorexigenic effect by inducing a decrease in the expression of the neuropeptides that induce appetite (*NPY/AgRP*).

Similar to insulin, another anorexigenic signaling pathway is activated by leptin[56,64]. Leptin is secreted from adipocytes in proportion to levels of body fat stores. Through the bloodstream it reaches first-order neurons, binds to its receptors and activates the Janus tyrosine kinase pathway and STAT3 pathway. STAT3 is a transcription factor that stimulates the expression of the precursor neuropeptide of α-melanocyte-stimulating hormone, named proopiomelanocortin (POMC) and the transcript regulated by cocaine and amphetamines (CART). These neuropeptides exert an anorexigenic effect[56,64]. Leptin and insulin signaling converge in the activation of PI3K/AKT, thus the anorexigenic effect is enhanced since the expression of *NPY/AgRP* is decreased by insulin and leptin, while POMC/CART expression is increased by leptin[56,64,65].

POMC/CART are the main anorexigenic neuropeptides expressed in neurons of the first-order (named neurons POMC/CART). These neurons release multiple cleavage products of POMC, including α-melanocyte-stimulating hormone, that bind in the second-order neurons located in the paraventricular nucleus, dorsomedial nucleus, ventromedial nucleus and lateral area nucleus to activate downstream melanocortin receptors (MC3R/MC4R) to promote satiety and control eating behavior, glucose homeostasis and body weight[54,58,64,66].

In periods of fasting, when glucose decreases, the release of insulin in the pancreas also decreases, and consequently the expression of POMC and CART decreases along with the satiety effect[56]. Meanwhile, the concentrations of ghrelin, a hormone secreted in the stomach during periods of starvation, increase[67]. This hormone reaches ARC through the bloodstream to activate the growth hormone receptor 1a, a G protein-coupled receptor, for the release of the α subunit from the βγ subunits of G protein. The α subunit activates phospholipase C. Phospholipase C induces the production of diacyl glycerol and phosphoinositol triphosphate. Phosphoinositol triphosphate is a second messenger

that binds to its receptor in the ER and causes the release of Ca^{2+} into the cytosol[68]. Increasing Ca^{2+} activates the Ca2+/calmodulin-dependent kinase II, which phosphorylates AMPK in the threonine 172 residue. AMPK activates transcription factors such as the cAMP-response element binding protein and forkhead box protein O1, which act on the promoter region of the *NPY* and *AgRP* genes, promoting their expression and inducing appetite[14,56].

NPY exerts its orexigenic effect on second-order neurons through stimulation of the Gi-coupled *NPY* family of receptors[66,69], mediating the inhibition of adenylate cyclase, decreased levels of cAMP[57, 70] and the activation of MAPK[61,70]. *AgRP* is a biased agonist of the melanocortin receptors (MC3R/ MC4R) and prevents the binding of α-melanocyte-stimulating hormone to these receptors, blocking the induction of satiety and driving sustained increase in food intake[66]. This constitutes an orexigenic signal.

Therefore, under normal physiological conditions, the release of the specific signal (inducing or inhibiting appetite) in the peripheral organs will depend on the metabolic state of the organism and will induce a response in the form of orexigenic or anorexigenic neurotransmitters in the hypothalamus[9,56, 64,66]. The strict regulation of these afferent and nutrient-related hormonal signals is necessary to avoid alterations in the regulation of appetite since an uncontrolled increase in POMC/CART would cause anorexia, but the uncontrolled increase in the expression of *NPY/AgRP* will generate hyperphagia, which due to excessive consumption of hypercaloric diets has been related to weight gain and obesity (characteristics that are linked to IR and T2DM)[9].

In studies in experimental models of hyperphagia and DM induced with streptozotocin, NPY/AgRP neurons are more active and the expression level of NPY and AgRP is increased, while POMC/CART neurons are less active and the expression level of POMC and CART is decreased. This change is explained in part to the inefficiency and/or deficiency of insulin[71,72] and leptin[73] and increased levels of circulating ghrelin[74,75].

During diabetic hyperphagia, high glucose intake will induce a proportional release of insulin from pancreatic β cells (hyperinsulinemia). The high concentration of insulin will induce the constant activation of the receptor at the cerebral and peripheral levels, which generates molecular and cellular regulation mechanisms such as: (1) Internalization of the receptor by clathrin-mediated endocytosis[76]; (2) Dephosphorylation in tyrosine residues of the insulin receptor by protein tyrosine phosphatase 1B, which is a nontransmembrane tyrosine phosphatase that acts as a potent negative modulator of insulin signaling by reversing insulin-induced phosphorylation in tyrosine residues and impairs insulin signal transduction; and (3) Phosphorylation on serine residues by serine-threonine kinases, such as JNK and the p38 MAPK[12,13]. This will generate a lack of response to the presence of the hormone (*i.e.* IR). At the level of the hypothalamus, this will decrease the activity of one of the pathways that induce satiety.

On the other hand, hyperphagia is often associated with the accumulation of visceral fat[77] and consequently elevated plasma leptin concentrations. This situation will induce the failure to respond to the hormone at central and peripheral levels, named leptin resistance[78,79]. In this way, there will be a decrease in the two central signals that induce satiety, favoring the persistence of hyperphagia and the onset of resistance to both hormones. This becomes a vicious circle: hyperphagia-hyperglycemia-hyperinsulinemia/hyperleptinemia-insulin/leptin resistance-hyperphagia.

In addition, excessive consumption of carbohydrates (glucose and/or fructose), coupled with a lack of physical activity, will generate an increase in glucose uptake in all cells but mainly in cells that have glucose transporters that act independently of the presence or absence of insulin[9,13,14] transporters, such as GLUT1 and GLUT3, mainly present in the brain[80]. With excessive intake of carbohydrates, glycolysis will increase, and therefore the release of ROS (species produced normally in glycolysis) will increase progressively until they overcome antioxidant barriers and oxidative stress develops[13,14,81].

It has been reported that during oxidative stress there is the activation of stress-sensitive kinases (JNK, p38 MAPK) that induce phosphorylation of serine residues in the insulin receptor and in the insulin receptor substrates, which blocks the pathway of insulin signaling aggravating the condition of IR[12,13]. In addition, studies carried out in rat models fed with fructose and subjected to an environ-mental stress protocol revealed that stress decreased body mass, adiposity and blood leptin level, decreased expression of the leptin receptor and POMC in the hypothalamus and led to a marked increase of AgRP, associated with AMPK phosphorylation and reduced Akt activity[14]. In parallel studies undertaken in normal rats, chronic blockade of hypothalamic insulin receptors caused hyperphagia and IR[82]. Furthermore, it has been reported that stimulation of hypothalamic insulin signaling would be sufficient to inhibit the glucose production in the liver through the intra-cerebroventricular administration of agonists and antagonists of insulin signaling[83], combined with evidence that mice with neuron-specific insulin receptor deletion are overweight, insulin-resistant and glucose-intolerant. These data demonstrate that neuronal insulin signaling is required for intact control of both body fat mass and glucose homeostasis[9]. Consequently, chronic stress can dysregulate the hypothalamus-adipose tissue[14,84] and hypothalamus-pancreas[64] axis over time, which affects glucose metabolism, promotes IR and influences multiple appetite-related hormones in the hypo-thalamus[64,84].

On the other hand, the effect of insulin has not only been studied in the hypothalamus at the level of glucose homeostasis. It has also been shown that the administration of insulin into the hippocampus of rats promotes Akt-dependent translocation of GLUT4[85]. Furthermore, hippocampal-specific
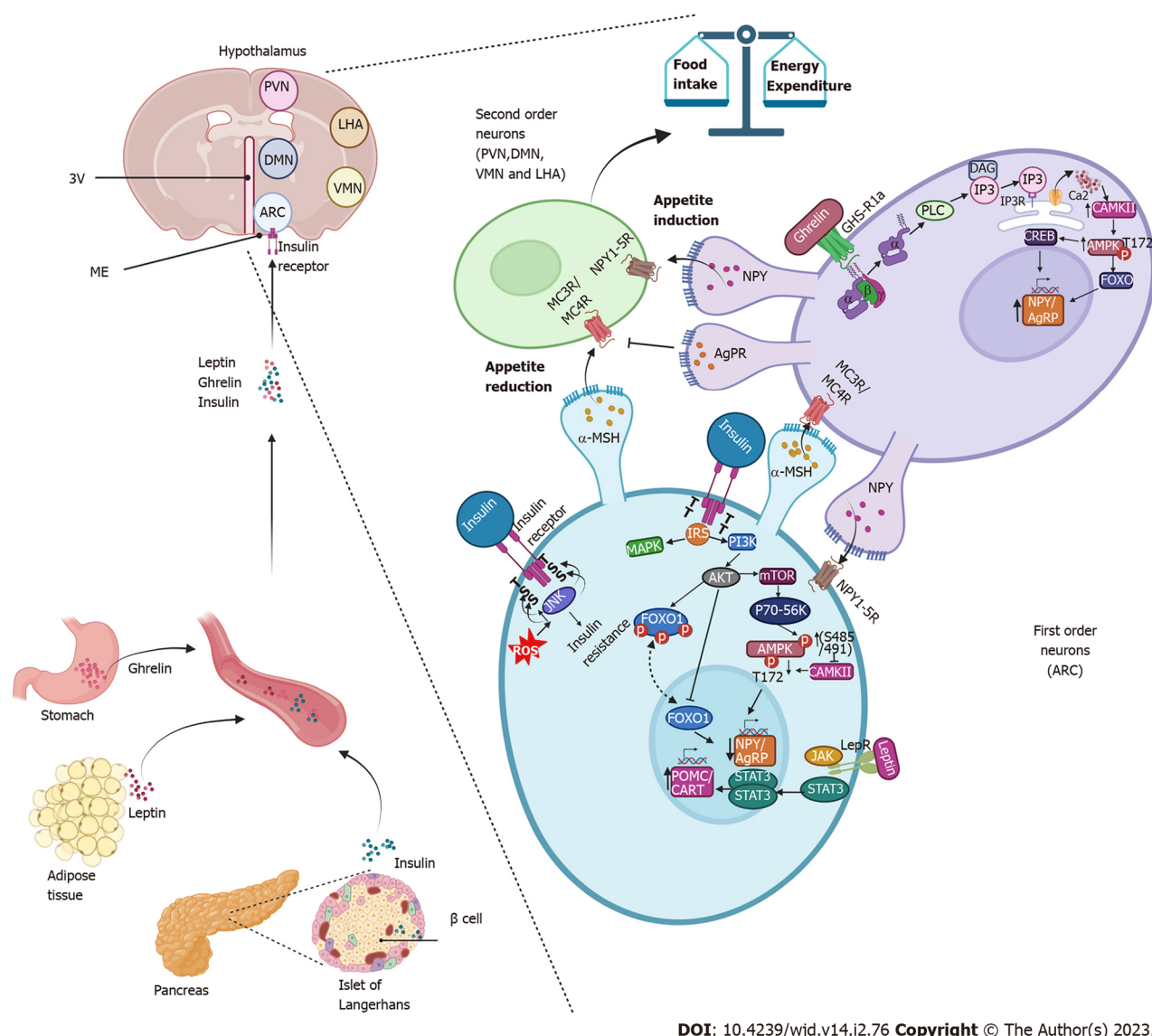
Figure 4 Insulin promotes decreased appetite in the arcuate nucleus. Insulin is secreted by the cells of the pancreas and through the circulation reaches the arcuate nucleus of the hypothalamus. It binds to its receptor on first-order neurons, triggering the phosphatidylinositol-3-kinase/protein kinase B signaling pathways and forkhead box protein O1 repression, resulting in decreased expression of neuropeptide Y *(*NPY*)* and agouti-related protein resulting in an anorexigenic effect. Like insulin, leptin activates anorexigenic signaling pathways by binding to its receptor, which activates the Janus tyrosine kinase/signal transducer and activator of transcription pathway, promoting the expression of the anorexigenic peptide precursor neuropeptide of α-melanocyte-stimulating hormone and transcript regulated by cocaine and amphetamines and with it the release of the α-melanocyte-stimulating hormone that activates the melanocortin receptors (MC3R/MC4R) in the neurons of the second order. Together, insulin and leptin signals amplify the anorexigenic effect. During fasting periods, ghrelin activates the growth hormone receptor 1a and promotes the activation of the adenosine monophosphate-activated protein kinase pathway that promotes the expression of *NPY*/agouti-related protein, stimulates orexigenic receptor Gi-coupled *NPY* in second-order neurons and prevents α-melanocyte-stimulating hormone from binding to melanocortin receptors (MC3R/MC4R), driving the orexigenic signal. 3V: Third ventricle; DAG: Diacylglycerol; IP3: Inositol triphosphate; IP3R: Inositol triphosphate receptor; ME: Median eminence; PLC: Phospholipase C; ROS: Reactive oxygen species.

suppression of insulin signaling reduces long-term potentiation in the hippocampus and significantly impairs memory and learning ability[86]. In hypothalamic neurons they have an important effect on body thermoregulation by signaling with brown adipose tissue[87]. Therefore, the effect of insulin at the brain level has been fully established. All cellular and molecular events are summarized in Figure 4.

THERAPEUTIC CONSIDERATIONS

Medical therapy is the first step to achieve adequate control of complications related to alterations in insulin secretion. Considering that DM is the main pathology related to this alteration, therapeutic treatments are focused on reducing hyperglycemia as well as stimulating the production and secretion of insulin in the cells of the pancreas and its signaling in the different tissues.

For T1DM, characterized by the destruction of the cells of the pancreas by autoantibodies as well as a decrease in the production and secretion of insulin, the first-line treatment is the administration of non-endogenous insulin[88]. Regarding T2DM, there are various therapeutic approaches, starting with improving eating habits[89] and increasing physical activity, which results in improving insulin sensitivity and helps control blood glucose[90]. When the above does not help control hyperglycemia, the therapeutic approach is based on the use of conventional drugs such as sulfonylureas (inducing insulin release from cells of the pancreas), biguanides (inducing glucose uptake by cells that are not insulin-dependent and reducing hepatic glucose production) and alpha-glucosidase inhibitors (blocking the absorption of glucose in the intestine)[91].

Currently, the use of incretin-based therapy has been implemented. Incretins are enteroendocrine hormones released after nutrient intake that stimulate glucose-dependent insulin secretion from β cells. To date, two incretins have been identified, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1. In mice, deficiencies in GIP and GLP-1 secretion are associated with decreased insulin response and impaired glucose tolerance. In this context, the overexpression of GIP or GLP-1 improves β cell function and glucose tolerance, and enhances insulin sensitivity. However, GIP also has an obesogenic effect, at least in animal models. Therefore, investigations have focused on GLP-1, specifically on its receptor. Agonists for GLP-1 receptor activation have recently been used. These include liraglutide, albiglutide, dulaglutide and semaglutide, and the results have been favorable for the management of DM[92].

On the other hand, the importance of finding new therapies that help improve disease control and the use of nutraceuticals has been increasing in recent years[93]. A positive effect has been reported in compounds such as melatonin[94], aloe vera extract[95] and hibiscus sabdariffa leaf extract[96]. They have regenerated pancreatic β cells and enhanced insulin secretion in streptozotocin-induced diabetic animal models. In patients with metabolic syndrome, a nutraceutical diet composed of barberine, policosanol, red yeast rice or tocotrienols significantly reduced the Homeostatic Model Assessment for IR index, leading to the conclusion that they have beneficial effects on IR[97,98].

Resveratrol, a polyphenol, found in many types of red fruits, has beneficial effects both *in vivo* and *in vitro*, showing great antioxidant capacity while improving insulin sensitivity[99,100]. Resveratrol is capable of activating the AKT pathway to stimulate insulin action[15]. The activation of sirtuin-1/AMPK has also been reported[101], which has a positive impact on mitochondrial biogenesis, inhibition of lipogenesis and fatty acid oxidation[102] and improves insulin sensitivity in DM[103,104].

Another antioxidant compound that has been less studied than resveratrol but with positive effects in models of obesity[105] and diabetes[106] has been curcumin, a non-flavonoid polyphenol[107]. In diabetic animal models, curcumin improves insulin sensitivity and increases glucose uptake. This mechanism is mediated by the liver kinase B1-AMPK pathway. Adding curcumin induced an increase in fatty acid oxidation, an event that improves insulin sensitivity[108]. At the brain level, curcumin increases glucose metabolism and improves the insulin signaling pathway, improving learning and memory[16] both under non-pathological conditions and in Alzheimer's disease[109]. Currently there are several studies on the use and beneficial effects of a wide variety of nutraceuticals, which are described in Table 1.

CONCLUSION

Insulin is a peptide hormone that plays an important role in various organs: in pancreas it participates in glucose homeostasis; in muscle it promotes glucose metabolism for energy generation and storage; in the vascular system it exerts an anti-atherogenic effect and participates in bone formation; in liver it decreases gluconeogenesis and favors glucose storage through glycogenesis; in adipose tissue it induces lipogenesis; and in brain it activates thermogenesis, regulates appetite, participates in glucose homeostasis and metabolism, reduces long-term potentiation and impairs memory and learning ability. Alterations in secretion or function of insulin considerably alter the cellular events regulated by the activation of its signaling pathway. Obesity and DM are pathologies associated with alterations in the function and secretion of insulin. In these pathologies, oxidative stress plays an important role since the uncontrolled increase in ROS derived from the increase in glycolysis due to the constant entry of glucose into the cells overcomes the antioxidant defenses. ROS induces alterations in insulin signaling and triggers a cascade of cellular alterations in various organs. Specifically in the hypothalamus, it can be the inducer of hyperphagia, which aggravates the diabetic condition and obesity. The use of antioxidants can be a complementary strategy to conventional treatment of DM.

Table 1 Mechanism of action of nutraceuticals

Nutraceutical	Mechanism of action	Study model	Ref.
Resveratrol	Reduces blood glucose and serum insulin levels, improves insulin and glucose tolerance, increases Sirt1, p-AMPK, p-IRS1 and p-AKT levels in liver	Mice, KKAy	[110]
	Enhances peripheral insulin signaling in diabetic mice in association with PTBP1 inhibition	Mice deficient in IRS2 (Irs2 -/-) and injected with STZ	[111]
	Reduces stress on the endoplasmic reticulum, thus improving insulin sensitivity and glucose levels	Mice C57BL/6J on a HFD	[112]
	Regulates protein expression of insulin receptor and GLUT4	Rat, Goto-Kakizaki	[113]
	Counteracts insulin resistance caused by hyperinsulinemia by activating AMPK and regulating GLUT4 translocation in muscle cells	L6 cell line	[114]
	Reduces insulin levels and the HOMA-IR index	Patients with T2DM	[115]
Curcumin	As a pretreatment, it protects pancreatic islets from cytokine-induced death	Mice, C57BL/6J	[116]
	Protects pancreatic islets from glycolipotoxicity by inhibiting oxidative stress and NADPH oxidase activity	Rats, Sprague-Dawley	[117]
	Improves insulin sensitivity and energy metabolism through the FNDC5/p38 MAPK/ERK pathways	Mice, C57BL/6J	[118]
	HOMA-IR index decreases	Patients with T2DM	[119]
Garlic	Decreases serum insulin level, HOMA-IR index and appetite	Patients with metabolic syndrome	[120]
Rhizoma polygonati odorati extract	Regulates serum insulin, adiponectin and leptin levels in mice on an HFD	Mice, C57BL/6	[121]
Diospyros kaki (persimmon) extract	Increases the number of pancreatic islets, decreases the expression of TNFα and IL-6, which interferes with insulin action	Zebra fish	[122]
Morus alba leaves	Decreases in the fasting insulin level and the HOMA-IR index, resulting in decrease of insulin resistance	Mice, C57BL/6 with HFD and STZ	[123]
Hydrolyzed pea protein	Enhances insulin-stimulated phosphorylation of AKT and FOXO1, increases IRS1 expression	Cells AML-12	[124]
Avocado oil	Improves insulin and glucose sensitivity	Mice, C57BL/6J	[125]
Eugenol	Improves glucose uptake in muscle, by insulin-independent pathway CaMKKβ/AMPK/GLUT4.	Mice, C57BL/6N with HFD and STZ	[126]
Okra leaf extract (*Abelmoschus esculentus*)	Regulates blood glucose level, food intake and changes in body weight	Wistar rats with STZ	[127]

AKT: Protein kinase B; AML-12: Alpha mouse liver 12 cells; AMPK: Adenosine monophosphate-activated protein kinase; CaMKKβ: Ca2+/calmodulin-dependent kinase β; FNDC5: fibronectin type III domain containing 5; ERK: Extracellular signal-regulated kinase; FOXO1: Forkhead box protein O1; GLUT4: Glucose transporter 4; HFD: High-fat diet; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; IL-6: Interleukin-6; IRS1: Insulin receptor substrate 1; MAPK: Mitogen-activated protein kinases; NADPH: Nicotinamide adenine dinucleotide phosphate reduced; PTBP1: Polypyrimidine tract-binding protein 1; Sirt1: Sirtuin 1; STZ: Streptozotocin; T2DM: Type 2 diabetes mellitus; TNFα: Tumoral necrosis factor alpha.

FOOTNOTES

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Diabetes and cognitive function: An evidence-based current perspective

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Abstract

Several epidemiological studies have clearly identified diabetes mellitus (DM) as a major risk factor for cognitive dysfunction, and it is going to be a major public health issue in the coming years because of the alarming rise in diabetes prevalence across the world. Brain and neural tissues predominantly depend on glucose as energy substrate and hence, any alterations in carbohydrate metabolism can directly impact on cerebral functional output including cognition, executive capacity, and memory. DM affects neuronal function and mental capacity in several ways, some of which include hypoperfusion of the brain tissues from cerebrovascular disease, diabetes-related alterations of glucose transporters causing abnormalities in neuronal glucose uptake and metabolism, local hyper- and hypometabolism of brain areas from insulin resistance, and recurrent hypoglycemic episodes inherent to pharmacotherapy of diabetes resulting in neuronal damage. Cognitive decline can further worsen diabetes care as DM is a disease largely self-managed by patients. Therefore, it is crucial to understand the pathobiology of cognitive dysfunction in relation to DM and its management for optimal long-term care plan for patients. A thorough appraisal of normal metabolic characteristics of the brain, how alterations in neural metabolism affects cognition, the diagnostic algorithm for patients with diabetes

and dementia, and the management and prognosis of patients when they have this dangerous combination of illnesses is imperative in this context. This evidence-based narrative with the back-up of latest clinical trial reviews elaborates the current understanding on diabetes and cognitive function to empower physicians to manage their patients in day-to-day clinical practice.

Key Words: Diabetes mellitus; Dementia; Cognitive function; Antidiabetic medications; Hyperglycemia; Hypoglycemia

Core Tip: Diabetes mellitus (DM) is a huge risk factor for cognitive dysfunction especially when the glycemic control is inadequate with marked hyperglycemia and recurrent hypoglycemia. Apart from cognitive decline inherent to the disease, presence of other forms of dementia can adversely affect diabetes control and consequently, negatively impact the care of dementia and DM. Appropriate control of DM with a multidisciplinary team approach involving diabetologists, dementia specialists, dieticians and physiotherapists should improve the clinical outcomes of either disease. Judicious and evidence-based adjustments in the antidiabetic medications appropriately tailored for individualised diabetes care with due consideration of patient's age, severity of dementia and other comorbidities should help to improve care of patients with diabetes and dementia.

INTRODUCTION

Diabetes mellitus (DM) has become a major cause of chronic disease morbidity in the past few decades, and according to the International Diabetes Federation data in the year 2021, approximately 537 million adults across the globe live with the disease[1]. DM can affect any organ system in the body, especially neural tissues and cerebrovascular structures causing various structural and functional disorders of the nervous system. Abnormalities in glucose metabolism including fasting and post-prandial hyperglycemia, prediabetic state and frank diabetes can result in neural dysfunction and various acute and chronic nervous system disorders including cognitive decline[2]. Cognitive dysfunction of chronic (and usually irreversible) nature that affects the usual intellectual performance of an individual is considered as dementia.

The World Health Organization (WHO) defines dementia as "a syndrome in which there is deterioration in cognitive function beyond what might be expected from the usual consequences of biological ageing". According to the latest estimates of WHO, more than 55 million people live with dementia, and about 10 million new cases added to this pool every year[3]. Although dementia often affects the elderly individuals, it is not an unavoidable consequence of biological ageing process. Dementia not only affects the physical, economic, and psychosocial functioning of the individual with the disease but also hugely impacts the carers, families, and the society, and therefore strains the healthcare systems at large.

Based on strong scientific evidence, DM is now identified as one of the major causes, and a potentially modifiable risk factor for the development dementia[2,4,5]. A recent meta-analysis of 122 studies observed that DM poses 1.25- to 1.91-fold higher risk for cognitive impairment and dementia[4]. The study also observed an elevated risk of dementia among subjects with prediabetes, fasting and postprandial hyperglycemia, elevated hemoglobin A1c (HbA1c) and those with abnormal fasting plasma insulin levels. Therefore, it is important to understand the pathobiology of diabetes and cognitive dysfunction to develop appropriate clinical algorithms for management of both the entities in day-to-day clinical practice which is the theme of discussion in this evidence-based review.

REVIEW METHODOLOGY

To compile most up-to-date and the best evidence on the topic of discussion, we performed a PubMed literature search to procure currently available best evidence. For this we used the MeSH terms/key words: "brain metabolism", "cognition/cognitive function", "cognitive dysfunction", "dementia", "memory loss/memory impairment", "diabetes mellitus" "type 2 diabetes mellitus/T2DM/T2D", "type

1 diabetes mellitus/T1DM/T1D", "pathobiology", "pathophysiology", "neuroimaging", "lifestyle intervention", "exercise", "diet", "antidiabetic medications", "insulin" "pharmacotherapy", "bariatric/ metabolic surgery", "prognosis", "clinical trials" and "diabetes technology".

The first two authors performed the initial literature search with guidance from the last two authors for initial drafting of the paper with an up-to-date search performed on 10th December 2022 for revising the paper after receiving the reviewer comments from the Journal. We used the Boolean search strategy using terms 'AND' or 'OR' where necessary to limit the search output to screen relevant abstracts from the web. We limited our literature review to articles published in English language. We used data and points from the most recent systematic reviews, randomised controlled trials (RCTs), clinical practice guidelines, and high-quality review articles to compile the best evidence available to us on DM and cognitive function to write the revision of this narrative review article.

ENERGY METABOLISM IN THE BRAIN

Although brain can use various metabolic substrates for energy production and utilisation, it predominantly uses glucose as the substrate for intermediary metabolism under normal physiological conditions[6,7]. The neuronal functions such as motor commands, sensory perceptions, memory storage, and intellectual output are highly dependent on the basal and on-demand metabolic activity of brain tissue. A graphical representation of normal neuronal glucose utilisation is shown in Figure 1.

Astrocytes, the supportive glial cells of the brain, normally take up glucose from circulating blood in the cranial arteries to provide energy substrate to brain for its neural functions and behavioral responses [8]. This astrocyte function as such is under the neuronal control through specific neurotransmitters and their receptors. Experimental animal models revealed that activation of such receptors (for *e.g.*, type-1 cannabinoid receptors associated with mitochondrial membranes in mouse astroglial cells) hampers the brain glucose metabolism with the production of lactate, resulting in alterations in the neuronal functions such as impairment of behavioral responses in social interaction assays[8]. These receptors are potential future targets for genetic and pharmacological manipulation for modulating such responses.

The energy metabolism of brain is highly variable in different areas depending on the neural functions and output of these regions. Most of the neural energy consumption is at the synaptic level for signal production and transmission along with the restoration of membrane potentials after depolarisation[9,10]. A good proportion of brain energy utilization is also for the synthesis of neurotransmitters, axoplasmic transport and the recycling of synaptic vesicles[10-12]. Overall, brain requires about 20% of the total oxygen and 20%-25% of glucose consumption of the body at rest, though the weight of human brain is only about 2% of the body weight[13-15]. However, during situations of stress and higher mental functions involving complex behavioral tasks, the metabolic demand increases further.

To facilitate optimal function of brain areas depending on the degree of neuronal output, supply-according-to-demand mechanisms have evolved through neurovascular and neurometabolic coupling for efficient substrate supply to the brain for fuelling intermediary metabolism[10]. Neurovascular coupling involves increase in blood flow and volume to improve glucose and oxygen supply to the areas of excess neuronal activity following stimulation, while neurometabolic coupling involves the changes in substrate utilization of astrocytes (predominantly by glycolysis) and neurons (predominantly by oxidative metabolism). These mechanisms are developed over centuries of genetic and metabolic adaptations in the evolution of the highly performing intellectual brain of modern man.

Metabolic adaptations of brain

As mentioned above, metabolic activity of the brain varies depending on its neural output for various biological tasks of daily life. Mitochondria are the powerhouses of brain's energy production as in other body cells, and therefore alterations in mitochondrial function can affect the intellectual performance of human brain in health and disease[15,16]. Mitochondria also functions as mediators of cellular "allostasis", a process of physiological adaptation of cells in response to various stressors[17]. By its bidirectional communication between stressors and stress mediators, mitochondria confer protective adaptive responses in the cells during period of acute stress[15]. "Neuronal plasticity", the physiological changes in neural electrical adaptive responses in response to various stimuli, is largely mediated through these metabolic adaptations at the mitochondrial level. However, chronic stressors of any category including alterations in glucose metabolism as observed in chronic hyperglycemia, hypoglycemia, and DM, can cause mitochondrial damage, resulting in neural dysfunction. These metabolic and nonmetabolic chronic stressors result in an "allostatic overload" causing mitochondrial dysfunction and various neurological disorders consequently[15,18].

Although glucose is the predominant energy substrate for human brain in physiological states, brain can use alternate fuels such as ketone bodies, lactate, and medium chain triglycerides when the body's glucose supply to brain is depleted as in periods of fasting and starvation[19,20]. This imply that the adaptive metabolic neural responses in relation to fasting and nutrient deprivation may have major biological impacts on brain function including cognition and intellectual performance. Recent evidence reveals that improvements in cognitive function, neuronal plasticity, and the resistance of brain to injury
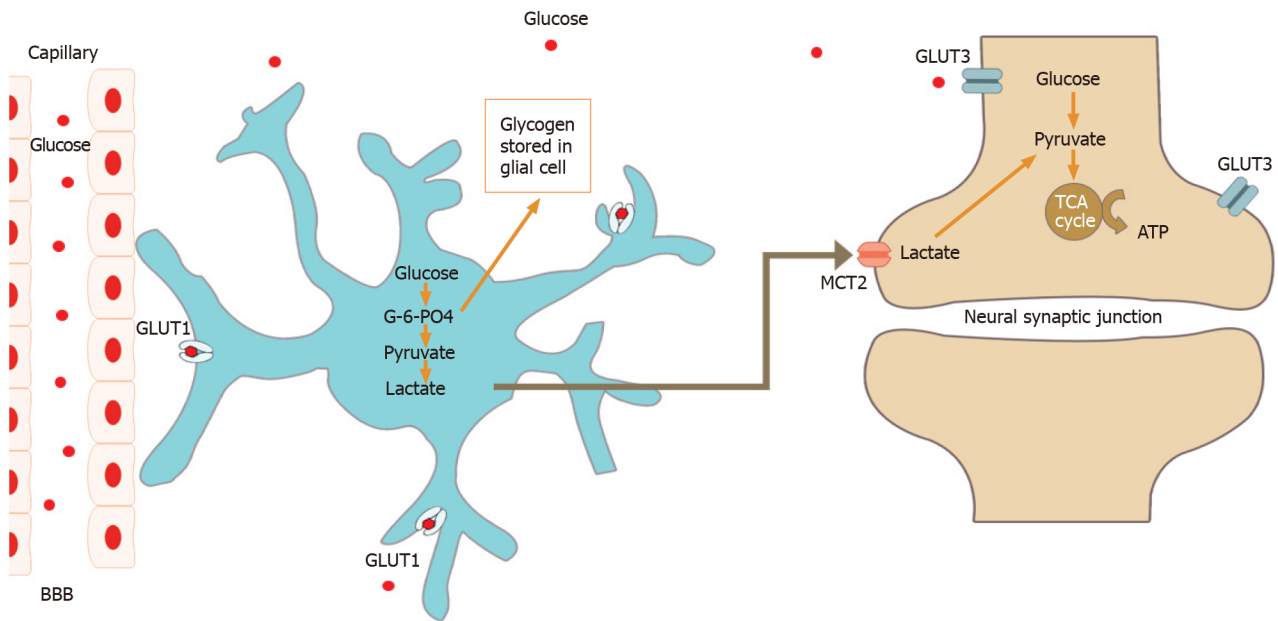
Figure 1 Neuronal utilisation of glucose under normal resting condition. G-6-PO4: Glucose 6-phosphate; BBB: Blood brain barrier; TCA: Tricarboxylic acid; GLUT: Glucose transporter; MCT2: Monocarboxylate transporter.

and disease occur in response to fasting and calorie restriction[20-22]. On other hand, over-fuelling as in metabolic disorders can result in disease states including neurodegeneration and dementia. Various changes in the central neural circuitry in response to dietary alterations may also modify the gut-brain axis which in turn alter the feeding behavior impacting the metabolic adaptation of brain as evidenced by recent scientific data[23].

BRAIN METABOLISM AND COGNITION

Intellectual capacities of human brain such as memory, mathematical performance, cognition, language, and executive functions are highly dependent on the degree of cerebral metabolic activity[10]. Therefore, any gross alterations in the metabolic milieu of brain are associated with marked changes in the neurocognitive balance in health and disease. Recent evidence suggests that there is a significant reduction in the glucose metabolism and functional connectivity between the intrinsic connectivity networks of brain with ageing, which would explain the age-related cognitive decline and decline in executive functions[24].

Lactate, another energy substrate of the brain, was also recently found to alter neurocognitive functions[25]. This by-product of intermediary metabolism was shown to increase transcription of brain-derived neurotrophic factor in neural cells and neuroglia. Lactate derived from "aerobic glycolysis" by astrocytes was found to enhance memory acquisition and learning-dependent synaptic plasticity in experimental mouse models[26] as shown in Figure 1. The energy demand of brain is often not adequately met by glucose supply from cranial circulation alone during exercise as glucose utilization by skeletal muscles increases substantially. In such situations, brain utilization of locally produced and muscle-derived lactate increases markedly to maintain metabolic demand for the enhanced neural synaptic activity[25].

Marked alterations in the metabolic activity of different areas of human brain is observed in various neurodegenerative disorders. For *e.g.*, in diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), and Lewis body dementia (LBD), the inferior parietal lobe was shown to have reduced glucose metabolism and perfusion defects[27-29]. These disorders are associated with significant reduction in cognitive function implying that the metabolic dysfunction has a contributory role in such cognitive decline.

DIABETES AND BRAIN DISORDERS

Glucose being the predominant metabolic substrate for the brain in normal physiological states, abnormalities in glucose homeostasis in diabetes is associated with marked changes in the structural

and functional alterations in the brain. Moreover, several brain areas such as hippocampus are very sensitive to local alternations in glucose metabolism inherent to diabetes which may result in neuronal synaptic reorganization[30], and augmented astrocyte proliferation[31]. These in turn can result in cognitive decline of diabetes especially because glucose and insulin are instrumental regulators of cognitive function[32].

There is a well-recognized association between higher glucose levels and the risk of dementia among individuals with or without diabetes as shown by Crane *et al*[33] in 2013. They observed an 18% higher risk of dementia at 5 years among subjects with a glucose level of 6.4 mmol/L compared to those with a glucose level of 5.5 mmol/L in nondiabetics while the risk of dementia was 40% higher among diabetics with glucose level 10.5 mmol/L compared to those with a level of 8.9 mmol/L. This study clearly demonstrates the linear relationship between higher ambient glucose levels in the central nervous system (CNS) and its potential long-term toxic effects on neurodegeneration resulting in dementia. Another study from the United States also showed a similar risk of cognitive decline (19% excess risk) among patients with diabetes at 20 years compared to nondiabetic individuals[34]. This large cohort study also revealed excess dementia risk among prediabetics, and the duration of abnormal glycaemia had an impact on the degree of cognitive decline in patients with diabetes.

Although the presumed genetic association between type 2 DM (T2DM) and AD (also known as type 3 diabetes) was recently refuted by a well-designed linkage analysis study[35], the two diseases appear to have a strong epidemiological link probably from a causal role of worsening AD in patients with diabetes[36-38]. The metabolic dysregulation within the CNS may accelerate the progression of AD and would explain this association. Even though LBD is found to have no direct association with diabetes [39,40], cognitive decline can be rapid in diabetics with LBD as these patients may not be on appropriate treatment[40]. Diabetes significantly increases the risk of vascular dementia (VaD) owing to the very strong association with cardiovascular disease (CVD), and stroke[41-44]. Regardless of the aetiology of dementia, care of diabetes and that of dementia can be challenging when these diseases co-exist especially in elderly individuals.

Pathophysiology central nervous system disease in diabetes

One of the putative mechanisms for cognitive dysfunction in T2DM is insulin resistance (IR) in the brain [45,46]. Neuronal cells express insulin receptors for its normal functions such as synaptic density and plasticity of dendrites[46,47]. Through various complex mechanisms, insulin receptor signaling improves synaptic and dendritic functions in the CNS to improve cognitive performance[46]. Therefore, central IR in T2DM is often associated with impaired cognitive function. The balance between central insulin sensitivity and IR have also been implicated in the feeding behaviour, satiety and development of obesity in experimental models[46,48,49]. Overnutrition and obesity, which usually lead on to T2DM, were found to be associated with disruption of the blood brain barrier leading to a state of neuroinflam-mation which in turn results in cognitive dysfunction[46,50,51]. Overnutrition also results in morpho-logical alterations in the hypothalamic neural circuitry that may augment overeating behaviour as a vicious circle aggravating obesity-related pathobiological states[46]. Alterations in gut microbiome commonly observed as part of the adverse eating habits are also associated with CNS neural changes causing cognitive decline[46,52].

Recurrent hypoglycemia is a common consequence of advanced diabetes especially those on insulin and sulphonylurea. Brain being an organ predominantly using glucose as its metabolic fuel, can have gross impact of hypoglycemic episodes especially when recurrent. The hypoglycemia awareness, partly evoked by neuroglycopenia, gradually diminishes as an adaptive response of recurrent hypoglycemia which will aggravate future risk of more severe hypoglycemic episodes and the consequent complic-ations[53]. Hypoglycemia-induced oxidative stress and neural inflammation can result in structural and functional alterations in vulnerable brain areas causing cognitive impairment[53-55]. Diabetes-induced vasculopathy affects the CNS circulation altering the cerebral blood flow remarkably. Both micro- and macrovascular damage involving the cranial vascular bed from accelerated atherosclerotic process inherent to diabetes are associated with neurocognitive decline and VaD[56-58]. Occurrence of microin-farcts and full-blown strokes are characteristics of longstanding diabetes[56]. Diabetes is identified as one of the most important causes of VaD mandating early diagnosis and proper management to reduce this potential consequence of the disease. A graphical representation of cognitive dysfunction in diabetes is shown in Figure 2.

DIABETES AND COGNITIVE DYSFUNCTION - DIAGNOSTIC EVALUATION

Appropriate diagnostic work up of cognitive dysfunction is especially important in patients with diabetes when compared to other medical problems as management of this metabolic disorder is largely patient-centred with regular glucose self-monitoring, and self-administration of medications including insulin injections. No other human disease needs such intense self-engagement as in diabetic patients for self-monitoring the metabolic parameters, medication compliance, dietary adjustments, and physical activities. Therefore, alterations in the mental functions can have a huge impact on diabetes control
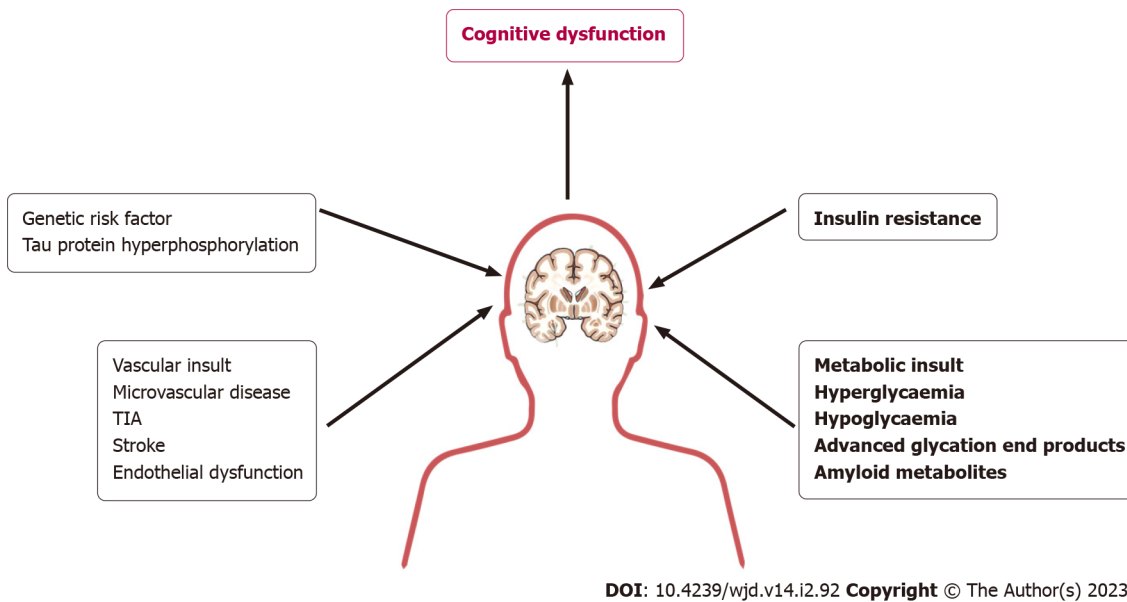
Figure 2 A graphical representation of the pathobiology of cognitive dysfunction in patients with diabetes. TIA: Transient ischemic attack.

which may further affect the cognitive balance in a vicious circle[59,60].

Biochemical evaluation

Periodic measurement of glycated HbA1c is the usual biochemical parameter that enable us to monitor long-term diabetes control in a patient with stable glucose levels without marked fluctuations in daily glycemia. HbA1c level reflects the average glycemic state over a period of 120 d, and therefore wouldn't always reflect good diabetes control in those with marked variability of glycemia as in patients having recurrent hypoglycaemia alternating with hyperglycaemia. Moreover, HbA1c levels can vary markedly in several conditions such as hemoglobinopathies, chronic kidney disease (CKD), anaemias and use of various medications[61]. Understanding these caveats of monitoring, appropriate use of HbA1c help us to get a reasonable measure of optimal diabetes management in patients with cognitive dysfunction.

If HbA1c is unreliable as in the situations mentioned above, an alternative biochemical test such as fructosamine test may be useful[61,62]. If there is an option for daily monitoring of capillary blood glucose (CBG) on multiple occasions, it provides the best chance of control of glycemia in patients with memory impairment[60]. Moreover, such monitoring would also enable us to optimise glycemic control. Newer glucose monitoring devices also enable calculation of predicted HbA1 levels which can be compared with the measured HbA1c to have idea about the reliability of the test.

Exclusion of other causes cognitive dysfunction such as thyroid disease, vitamin deficiencies and liver disorders by appropriate biochemical and hormonal evaluation is mandatory as part of initial evaluation and follow up care as and when necessary. As these diseases can often co-exist in some patients with diabetes, prompt testing would help timely diagnosis and appropriate management.

Neuroimaging

Neuroimaging is an integral part of routine initial evaluation of cognitive dysfunction in any individual to exclude structural abnormalities of the brain. Again, when there is a rapid unexplained decline in cognition without a clear identifiable reason in patients with known dementia, neuroimaging is warranted to exclude such abnormalities. Even minor unnoticed trauma can be associated with intracranial bleeds in elderly individuals which can be associated with rapid decline in memory function indicating urgent neuroimaging. Amyloid angiopathy is another disorder associated with spontaneous intracerebral bleed which may present similarly with an indication for urgent imaging studies[63].

Computed tomography scan is the usual first line imaging modality in most centres as it is cost effective, easily available, and provides reasonable sensitivity for initial evaluation of most major structural lesions such as stroke, tumors and hematomas[63-65]. Magnetic resonance imaging and positron emission tomography may be necessary for further evaluation of patient's with cognitive dysfunction for accurate diagnosis of the pathological entity and for follow up management[66,67]. In the evaluation and follow up of patient's with diabetes and cognitive dysfunction, imaging studies are indicated to exclude the possibility of development of such structural abnormalities described above or the co-existence of other disease entities such as AD, LBD or VaD.

MANAGEMENT OF DIABETES IN DEMENTIA

Owing to the risks associated with hypoglycemia in dementia patients, tight glycemic control is not usually recommended as in a patient without memory problems. Acceptable glucose and HbA1c targets are usually set by healthcare providers depending on the degree of cognitive impairment and other associated co-morbidities in the patient. Patient's ability for CBG monitoring, and antidiabetic medication self-administration should be assessed promptly on periodic basis to optimise glycemic management. Individualised glycemic targets should be set with due consideration of patient's situation and comorbid illnesses such as CKD, heart disease and hypoglycemia awareness. If self-care of diabetes is an issue, ensuring of regular assisted care by family members or by care providers becomes essential. In situations where these are not feasible, institutionalised care is recommended.

How diabetes management impacts dementia

Optimal diabetes care was found to be associated with better cognitive outcomes in patients with established dementia based on the data from multiple studies[68-70]. As discussed earlier, the glycemic load and IR in areas of brain associated with processing, storage and retention of memory has impact on cognition and therefore, optimising glycemic management may have significant influence on prognosis of patients with dementia. Prevention of marked hyperglycemia with appropriate adjustments of glycemic management should be tailored to suit the individual requirements of the patient on periodic basis to achieve this goal. While attempting to prevent marked hyperglycemia, all necessary precautions should be taken to prevent hypoglycemic episodes which can negatively impact on neurocognitive function[70,71]. Therefore, immediate and long term targets on clinical and biochemical parameters should be set periodically for glycemic and diabetes control in every patient with established cognitive dysfunction.

Dietary management

Dietary adjustment to optimise adequate nutritional supply while avoiding marked glycemic fluctuations is the corner stone of management of any form of diabetes in patients with the disease. This principle is equally important in patients with diabetes and dementia as nutritional deficits may have a negative impact on neurocognitive outcomes whereas appropriate nutritional interventions may have beneficial effects[72]. Low carbohydrate, high fibre diets with proteins and fat in moderation may be entirely appropriate to dementia patients as in normal subjects though palatability and refusal of timely intake of food can pose problems especially in advanced stages of the illness. A diet plan with due consideration of the sociocultural factors should help to improve adherence to such dietary interventions in cognitively impaired individuals with DM as in normal diabetic patients[73].

Physical activities

Regular moderate intensity physical activity is an integral part of daily management of any individual with DM. Physical activity improves skeletal muscle metabolism which in turn reduces the IR and insulin sensitivity and therefore improves diabetes and cardiometabolic parameters. As most patients with dementia are older individuals, exercise interventions may also improve sarcopenia associated with old age[74,75]. Such interventions improve the cognitive function and also reduce risk of imbalance of ageing and consequent falls. As the metabolism improves with exercise interventions, the diabetes management regime needs to be periodically revisited to avoid the risk of hypoglycemia. Multiple studies clearly demonstrated the remarkable benefits of exercise interventions on long term diabetes control, cognitive functions, and even risk of hypoglycemia in patients with dementia[76-78]. Therefore, an appropriate physical activity program should be considered for all patients with dementia to optimise the management with due consideration of the exercise capacity, co-morbidities and patient cooperation.

Optimising drug therapy

All patients with type 1 DM (T1DM) at onset of the disease, and most patients with T2DM at some stage of the disease would require pharmacotherapy for management of hyperglycemia. Insulin treatment is an absolute requirement for patients with T1DM from the diagnosis whereas many patients with T2DM are largely managed by noninsulin pharmacotherapy. Compliance with medical management can be a major issue in dementia patients with marked memory impairment complicating diabetes care. Therefore, healthcare providers' responsibility is greater in managing such patients while ensuring adequate glycemic care with the avoidance of over-/undertreatment associated with significant morbidity and even mortality risks.

Insulin and insulin secretagogues (*e.g.*, sulphonylurea and meglitinides) can be associated with significant risk of hypoglycemia while combination treatment of these with other molecules such as metformin, dipeptidyl peptidase-4 inhibitors (DPP-4i) and glucagon like peptide-1 receptor agonists (GLP-1RA) may potentiate the risk of hypoglycemia. The use of latter two molecules may have a beneficial effect in slowing down the cognitive decline in patients with dementia as revealed by a recent meta-analysis[79]. A detailed appraisal of treatment with individual antidiabetic agent in managing

patients with dementia is beyond the scope of this review and therefore, readers are recommended to follow standard guidelines with consideration of individual patient characteristics based on the broad principle of avoiding hypoglycemia while optimising glycemic control.

Bariatric surgery

Bariatric procedures are associated with massive improvements in obesity and are the best available treatment modality for patients with obesity especially when associated with comorbidities such as T2DM. A significant proportion of patients achieve remission or reversal of T2DM. Metabolic surgery has been found to be associated with remarkable improvements cognitive function in patients with memory deficits in various observational studies[80-82]. However, with the currently available data, it is difficult to make firm recommendations in the absence of large scale long term follow up data based on RCTs.

Impact of dementia on diabetes care: Gradual decline in the memory deficits over time is the usual long-term consequence of all forms of irreversible dementia. Worsening memory is expected to have a huge impact on diabetes care especially when patients self-manage their diabetes. Medication compliance issues with inappropriate meal timings and improper administration of antidiabetic medications can adversely affect glycemic care with further decline in memory function. The resultant fluctuations in glycemia with uncontrolled hyperglycemia and recurrent hypoglycemic episodes will worsen diabetes-related complications and cause rapid decline in the neurocognitive functions[83].

Medication compliance

Memory impairment is usually associated with a decline in the executive functions of day-to-day living such as self-care, nutritional intake, and monitoring of medical problems such as diabetes early in the course of dementia. Forgetfulness associated with inadequate drug intake is common in patients with cognitive dysfunction and computer assisted cognitive training is shown to improve diabetes self-care in such patients[84]. When compliance issues become marked with poor diabetes self-management and recurrent acute hyperglycemic complications, home-based or institutionalised care support should be considered for supervised glycemic management.

Hypoglycemia management

Improper administration of antidiabetic agents such as insulin and insulin secretagogues without timely food intake can result in marked hypoglycemic episodes which can result in falls, rapid decline in cognitive functions and even death. Prompt review of diabetes drug regime with appropriate changes in the pharmacotherapy should be enforced urgently in such situations. Multidose insulin regime may need to be switched over to once daily long-acting insulin or twice daily mixed insulin regimes may be considered with due consideration of patient's diet and physical activities[85].

Discontinuation of insulin secretagogues with hypoglycemic potential also need to be considered in presence of erratic meal pattern of patients with moderate to severe dementia[85,86]. Antidiabetic agents with less propensity for hypoglycemia and drugs which need less frequent administration such as DPP-4i and GLP-1RA are preferable in such situations. Although sodium-glucose cotransporter-2 inhibitors are hypo-neutral agents, the use of these agents in patient with advanced dementia needs caution as these patients can get dehydrated due to diuretic effect of these agents. Supervised drug administration by carers and institutionalised care should be considered to improve medication adherence and glycemic care in patients with advanced dementia.

PROGNOSIS OF DEMENTIA IN DIABETES

Prognosis of patients with dementia largely depends on the type and the pathobiology of the individual disorders causing cognitive dysfunction. However, prompt diabetes care may alter the course of the disease to some extend because of the impact of altered glucose metabolism on brain structures as mentioned in the previous sections. There is some emerging evidence showing beneficial effects of treatment with antidiabetic medications of the GLP-1RA class for neuroprotection in patients with PD, AD, stroke, and amyotrophic lateral sclerosis[87]. Moreover, optimal diabetes management may help to prevent deterioration of cognitive function in various dementing illnesses in relation to hypo- and hyperglycemic complications of improper diabetes care.

Diabetes types and dementia

Dementia may occur in patients with any of form of diabetes regardless of the type. The degree of cognitive decline in such patients largely depends on the appropriateness of diabetes management as mentioned earlier. As care of both the disorders can impact the management and prognosis of the other, healthcare providers are expected to have good understanding of either disease pathobiology. A multidisciplinary team (MDT) approach involving dementia specialists, physiotherapists, diabetologists and dieticians may help to optimise management of patients with moderate to severe forms of either

disease. Moreover, individualised care plans for patients with consideration of their age, sociocultural factors, and comorbidities are important to obtain optimal outcomes.

T1DM and dementia

Patients with longstanding T1DM are at risk of some form of cognitive impairment, and diabetologists caring for such patients should be vigilant in identifying incident cognitive dysfunction in such patients. As a significant proportion of T1DM cases are on basal bolus insulin regimes (once/twice daily long-/ intermediate- acting insulin and mealtime short acting insulin), compliance issues with timing of meals and insulin administration may emerge as serious problems early in the course of dementia. Rapid decline of diabetes control with adverse consequence on cognitive functions are the results of such a situation[88].

Regular CBG monitoring is important in the management T1DM patients to aid variable dose mealtime insulin administration adjusted for their carbohydrate intake. Recently, intermittent scanning of a continuous glucose monitoring device (measuring interstitial tissue glucose) has revolutionised the self-monitoring of glycemic parameters and diabetes care in such patients[89]. Appropriate use of such technology in a supervised setting can potentially mitigate the cognitive decline in relation to poor glycemic care in patients with dementia. Appropriate changes in the insulin regime as mentioned earlier also may be necessary in patients with poor meal compliance and insulin administration issues.

T2DM and dementia

There is some evidence to support the notion that AD may have an association with T2DM based on multiple epidemiological correlation studies[90-92]. Although the pathobiological interlink is not very strong, we have to consider this association while planning management of patients with T2DM, especially because of the constraints imposed on glycemic care by the development of dementia. Appropriate early administration of medications of incretin mimetic class such as DPP-4i and GLP-1RA to optimise diabetes control and prevention of AD will help to some extent[87,93]. Although there has been a signal towards some vague association of metformin use to the development of AD in Asians in a recent meta-analysis[94], the study results have to be interpreted with caution as the data analysed was of low quality and of observational type. Insulin administration issues can be addressed as mentioned earlier.

Other types of diabetes and cognitive function

There is not much data on the incidence and prevalence of cognitive dysfunction in patients with other forms of diabetes such as diabetes in patients with chronic pancreatitis, monogenic diabetes, and syndromic type of diabetes. However, glycemic care can pose similar problems when cognitive decline becomes moderate to severe as in T1DM and T2DM. Nutritional imbalance from pancreatic diabetes and neurological problems in some patients with syndromic diabetes can pose problems in glycemic care. Supportive care with an appropriate MDT approach might help to improve care in such patients.

USE OF NEWER DIABETES TECHNOLOGIES FOR THE CARE OF PATIENTS WITH COGNITIVE DYSFUNCTION

Regular monitoring of CBG can be hectic and add additional burden to patients with dementia. Although use of flash glucose monitoring device can avert the finger pricking, patients with dementia can forget flashing their device resulting in loss of data if not scanned for more than eight hours. The real-time continuous glucose monitoring system (rtCGM) offers benefit in automatically sensing and transmitting the data to the application on the phone. Wireless Innovation for Seniors with Diabetes Mellitus trial compared rtCGM with standard finger prick capillary glucose monitoring in older adults (age > 60 years) with T1DM for prevention of hypoglycaemia and glycaemic control[95]. This trial also included patients with mild cognitive impairment although individuals with advanced dementia were excluded. The rtCGM arm spent less time below the range (blood glucose < 70 mg/dL) and there was also significant reduction in the HbA1c in the rtCGM arm when compared with the control (mean difference of -0.3%; 95% confidence interval: -0.4% to -0.1%; P < 0.001). With the lesson learned during coronavirus disease 2019 pandemic with rtCGM and third party data sharing, these features can help the family members, carers or care givers monitor the glucose level remotely and help the patient with the decision regarding insulin dose calculations[96,97].

Appropriate use of technology can help to manage T1DM and insulin treated T2DM individuals when they develop dementia. Although there is no robust evidence through RCTs, sensibly matching the technology to the individual needs and support system will ease the management[98]. Disposable insulin pens reduce the workload for the patient than the reusable pen that needs periodic change of the cartridge. The use of smart insulin pen automatically uploads the delivered dose in the linked server [99]. The alarm features in these pens can be an additional advantage to remind the timely adminis-tration of insulin. Remote review of the doses by the carer can help with titrating the dose, deliver the

missed dose and prevent overdosing with insulin. Individuals with mild dementia do manage the insulin pump with ease if they are used to it for a long duration before the dementia settles[100]. The insulin pump with predictive low glucose suspend features can help preventing hypoglycaemia provided that patient does not pull out the pump connections, hence not to be used in patient with moderate to advanced dementia as the risk of diabetic ketoacidosis will be high if there is disconnection. The behaviour of patient with the insulin pump can be studied using saline filled cartridges in the practice for a period of a week or two, and the information derived can help to decide about the individual's ability to manage the insulin pump.

If the individual manages the insulin pump, then use of hybrid closed loop insulin delivery system can be tried as it can vary insulin basal delivery depending on the blood glucose level rather than the set basal targets and the trials have clearly shown beneficial effects in elderly patients with T1DM[100]. The remote blousing feature with smart phone in some of the hybrid closed insulin technology (*e.g.,* CamAPS FX hybrid closed loop app) will help carers in delivering the correct insulin dose. This third-party insulin delivery *via* the remote blousing feature should only be used in the countries where such regulation is allowed. Lastly, the use of insulin only bionic pancreas where only qualitative announcement of the meal is required can be an additional tool for management of individual with mild early dementia where complicated carbohydrate counting can be ignored[101]. More trials using technology in patients with early dementia are needed, as there is an increase in elderly patients with T1DM and it is predicted that prevalence of T1DM itself will be doubled by the year 2040[102].

OTHER COMORBIDITIES/MODIFIABLE RISK FACTORS POTENTIALLY IMPACTING COGNITIVE DYSFUNCTION IN DIABETES

Several other coexistent illnesses can exaggerate the risk of cognitive decline in patients with diabetes. Therefore, management of these comorbidities are also very crucial for optimal long-term outcomes.

Hypertension

One of the most common chronic diseases affecting middle-aged and the elderly population is hypertension. It is one of the most common comorbidities in the diabetic populations, especially in those with early onset T2DM with a prevalence of about 67.5%[103]. A recent study showed that the odds ratio for dementia in patients with hypertension is 5.82, and more than 90% dementia patients with diabetes had hypertension[104]. From this observation, it is imperative to obtain prompt blood pressure (BP) control in patients with dementia while considering the risks associated with intense BP reduction such as postural hypotension and falls. Antihypertensive medications modifying the renin-angiotensin-aldosterone system have been recently found to improve executive function, processing speed, verbal memory and composite score compared to other antihypertensive medications in a recent clinical trial [105].

Dyslipidemia

Several studies have shown association between dyslipidemia and dementia especially when present in patients with diabetes[106]. Diabetes (especially T2DM) as such is a strong risk factor for atherosclerotic CVD even in patients with normal lipid levels for nondiabetic individuals. This may be related to presence of more atherogenic low-density lipoprotein cholesterol particles in diabetics making them prone to develop CVD. Accelerated atherosclerosis of the cranial arteries may be an important factor reducing cerebral blood flow and cognitive decline in such patients. However, intense lipid lowering therapy was not associated with better cognitive outcomes in the ACCORD clinical trial[107].

Associated CVD

CVD is an important risk factor for dementia owing to its close association with cerebrovascular disease, stroke, and impaired brain perfusion. Even in those without established cerebrovascular disease or stroke, CVD was found to be associated with higher rates of cognitive decline in a systematic review [108]. The authors observed that severe atherosclerosis posed 59% and atrial fibrillation posed 26% higher risk for development of dementia.

Proteinuria

Both micro- and macroalbuminuria are associated with high risk of generalised vasculopathy and atherosclerotic disease. Diabetes-related microvascular disease affects kidneys early in the course of the disease, especially in patients with poor diabetes control, and tremendously exaggerate the athero-sclerotic CVD. A recent meta-analysis showed significant association between albuminuria and cognitive dysfunction[109]. This systematic review involving 16 studies among 127296 participants revealed a 20% excess risk of dementia among patients with albuminuria.

Table 1 Risk factors for cognitive decline in diabetes mellitus

Risk factors for cognitive decline in diabetes mellitus	
Age > 60 yr	Atherosclerotic cardiovascular disease
Presence of ApoE ε4 allele	Uncontrolled hypertension
Long duration of diabetes	Proteinuria
Poor glycaemic control/high HbA1c	Dyslipidaemia
Higher fasting glucose levels	Physical inactivity
Recurrent hypoglycaemic episodes	Unhealthy diet
Severe insulin resistance	Depression

ApoE: Apolipoprotein; HbA1c: Hemoglobin A1c.

Table 2 Landmark randomized controlled trials looking at the benefits of clinical management of modifiable risk factors in diabetes and cognitive dysfunction

| Intervention/ treatment	Study characteristics & benefit(s) of treatment/intervention group	Ref.
Treatment with antihypertensives acting on renin angiotensin axis	Better executive function, processing speed, verbal memory and composite score compared to those treated with other antihypertensives	Wharton *et al* [105], 2022
Intensive BP and lipid control compared to standard treatment (ACCORD trial)	Intense BP control and lipid reduction had no effects on cognitive decline. Moreover, total brain volume was found to be less with intense BP control (systolic BP < 120 mm Hg) than standard treatment after 40 mo	Williamson *et al* [107], 2014
Liraglutide therapy for T2DM	Activation of different cerebral areas with improved memory, attention, and better scores in all cognitive function tests	Li *et al*[112], 2021
Intense *vs* standard BP control (SPRINT trial)	Intense BP control was not associated with improvements in memory or processing speed compared to standard BP reduction	Rapp *et al*[113], 2020
10 yr of ILI *vs* standard care (Look AHEAD trial)	ILI resulted in better odds for emergence of: Decision-making inability (OR = 0.851) and problem solving inability (OR = 0.694) in those without these baseline complaints	Espeland *et al* [114], 2018
Finnish diabetes prevention study	Middle-aged overweight participants with impaired glucose tolerance showed better cognitive performance with low total fat & saturated fat intake, and frequent physical activities compared to standard lifestyle	Lehtisalo *et al* [115], 2016

BP: Blood pressure; T2DM: Type 2 diabetes mellitus; ILI: Intense lifestyle intervention; OR: Odds ratio.

Apolipoprotein ε4 allele

Apolipoprotein (ApoE) is protein that carries the lipid molecules for their transport in human body in the form of apolipoproteins. Historically, career of ApoE ε4 allele has been found to possess strong association with the development of dementia[110]. A recent study involving 206960 participants from the United Kingdom biobank cohort showed that the presence of ApoE ε4 allele was associated an increased risk [hazard ratio (HR) = 1.63] of developing dementia[111]. However, when potentially modifiable risk factors such as hypertension, diabetes and coronary artery disease were clustered in to this risk, the HR increased to 2.20. Table 1 summarises the risk factors for dementia or cognitive decline among patients with diabetes.

CLINICAL TRIALS ON MODIFIABLE RISK FACTORS OF COGNITIVE DYSFUNCTION AMONG PATIENTS WITH DIABETES

Several RCTs examined the potential benefits of management of various modifiable risk factors for cognitive decline in patients with diabetes. However, only a small proportion of these studies showed even marginal benefits. Some of the trials even showed the probability of harm in the participants. Therefore, we need much more research input in this area to ensure we have more promising modalities of treatment for diabetic patients with cognitive dysfunction. A list of landmark clinical trials looking at the benefits of potentially modifiable risk factors for managing patients with diabetes and cognitive dysfunction is shown in Table 2.
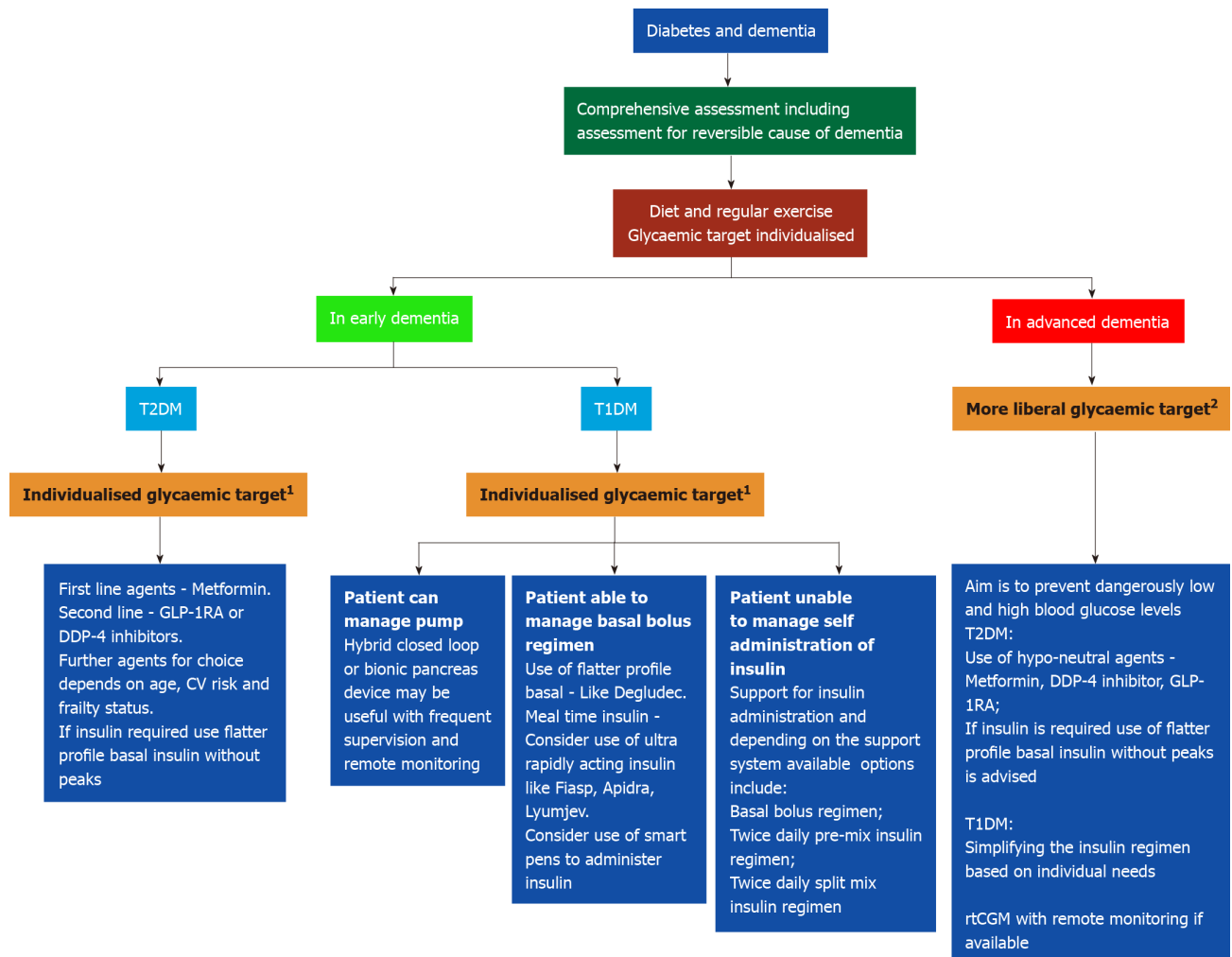
Figure 3 Practical approach to the management of patient with diabetes and dementia. [1]Glycaemic target according to comorbidities to avoid marked glycaemic variability, hypo- and hyperglycaemia. [2]Target glucose 7-12 mmol/L ideally (but can range between 5-16 mmol/L especially while on insulin). T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; GLP-1RA: Glucagon like insulinotropic peptide-receptor agonist; DPP-4: Dipeptidyl peptidase-4; CV: Cardiovascular; rtCGM: Real-time continuous glucose monitoring.

AREAS OF UNCERTAINTY/EMERGING CONCEPTS

Although optimal glycemic care is expected to ameliorate the cognitive decline associated with hyper- and hypoglycemic care of patients with diabetes and dementia, it is not clear if prompt diabetes control might alter the pathobiology of individual dementing illnesses. The proposed association between T2DM and AD is currently vague, and more studies may shed light on this grey area.

The benefits of observed improvement of cognitive function among patients with massive weight loss following bariatric surgery need additional evidence through largescale RCTs for use in day-to-day clinical practice. The beneficial effects of incretin manipulation by GLP-1RA and DPP-4i on different forms of neurodegenerative disorders such as AD need to be clarified in long term RCTs. The potential risk of metformin use and AD development revealed in some ethnic groups needs further studies as metformin is the first-line drug with other remarkable health benefits when used in patients with T2DM. Figure 3 shows a pragmatic approach to the management of diabetes and dementia in day-to-day clinical practice.

CONCLUSION

Development of cognitive dysfunction is a big risk of inadequate diabetes management in patients with any form of diabetes. Onset of dementia can impact diabetes care with the risk of worsening of either disease from inadequate glycemic care. Currently available evidence suggest that optimal diabetes management can have better clinical outcomes among patients with neurocognitive dysfunction. A multidisciplinary approach to management of patients involving diabetologists, dieticians, dementia

specialists and physical therapists with appropriate antidiabetic treatment and nonpharmacological interventions may improve diabetes care in patients with diabetes and dementia. If appropriately used, technological advancements can further improve the care of diabetes patients with dementia. More research is needed in these areas as the incidence of both the diseases are increasing globally owing to increasing prevalence of obesity and aged individuals in the global population.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Protective effect of liraglutide on the myocardium of type 2 diabetic rats by inhibiting polyadenosine diphosphate-ribose polymerase-1

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Abstract

BACKGROUND

In recent years, studies have found that the occurrence and development of diabetic cardiomyopathy (DCM) is closely related to an increase in polyadenosine diphosphate-ribose polymerase-1 (PARP-1) activity. PARP-1 activation could be involved in the pathophysiological process of DCM by promoting oxidative stress, the inflammatory response, apoptosis and myocardial fibrosis.

AIM

To investigate the mechanism of liraglutide in improving myocardial injury in type 2 diabetic rats, further clarified the protective effect of liraglutide on the heart, and provided a new option for the treatment of DCM.

METHODS

Forty healthy male SD rats aged 6 wk were randomly divided into two groups, a normal control group ($n = 10$) and a model group ($n = 30$), which were fed an ordinary diet and a high-sugar and high-fat diet, respectively. After successful modeling, the rats in the model group were fed a high-glucose and high-fat diet for 4 wk and randomly divided into a model group and an intervention group (further divided into a high-dose group and a low-dose group). The rats were fed a high-glucose and high-fat diet for 8 wk and then started drug intervention. Blood samples were collected from the abdominal aorta to detect fasting blood glucose and lipid profiles. Intact heart tissue was dissected, and its weight was used to calculate the heart weight index. Haematoxylin and eosin staining was used to observe the pathological changes in the myocardium and the expression

of PARP-1 in the heart by immunohistochemistry.

RESULTS
The body weight and heart weight index of rats in the model group were significantly increased compared with those in the normal control group, and those in the intervention group were decreased compared with those in the model group, with a more obvious decrease observed in the high-dose group ($P < 0.05$). In the model group, myocardial fibers were disordered, and inflammatory cells and interstitial fibrosis were observed. The cardiomyopathy of rats in the intervention group was improved to different degrees, the myocardial fibers were arranged neatly, and the myocardial cells were clearly striated; the improvement was more obvious in the high-dose group. Compared with the normal control group, the expression of PARP-1 in myocardial tissue of the model group was increased, and the difference was statistically significant ($P < 0.05$). After liraglutide intervention, compared with the model group, the expression of PARP-1 in myocardial tissue was decreased, and the reduction was more obvious in the high-dose group ($P < 0.05$) but still higher than that in the normal control group.

CONCLUSION
Liraglutide may improve myocardial injury in type 2 diabetic rats by inhibiting the expression of myocardial PARP-1 in a dose-dependent manner.

Key Words: Liraglutide; animal models; Type 2 diabetic rats; Polyadenosine diphosphate-ribose polymerase-1; Haematoxylin and eosin staining; Immunohistochemistry

Core Tip: Low-dose streptozotocin combined with a high-glucose and high-fat diet can successfully establish a rat model of type 2 diabetes mellitus. After 4 wk of continuous feeding, myocardial injury can occur, which is consistent with diabetic cardiomyopathy. Liraglutide reduced the body weight of type 2 diabetic rats and significantly improved the fasting blood glucose and lipid profile in a dose-dependent manner. Liraglutide may improve myocardial injury in type 2 diabetic rats by inhibiting the expression of myocardial polyadenosine diphosphate-ribose polymerase-1 in a dose-dependent manner.

INTRODUCTION

Diabetic cardiomyopathy (DCM) is a specific type of cardiomyopathy independent of hypertension, coronary heart disease, and congenital heart disease. The early manifestation of DCM is reduced left ventricular diastolic function, which can be combined with end-stage systolic dysfunction, increasing the risk of heart failure in patients with diabetes. Diabetes seriously affects the patient's life, and its incidence is increasing year by year. Its specific pathogenesis is complex and has not been fully elucidated[1-3]. Polyadenosine diphosphate ribose polymerase-1 (PARP-1) is a nuclear protein widely present in the nuclei of most eukaryotes. After activation, PARP-1 catalyzes nicotinamide adenine dinucleotide (NAD+) to form a poly-adenosine diphosphate ribose (PAR) chain and glycosylate and regulate the functions of histones, topoisomerases, DNA polymerases, *p53*, nuclear transcription factor (NF-κB) and other proteins, which are involved in DNA damage repair and other cellular functions. In recent years, studies have found that the occurrence and development of DCM is closely related to an increase in PARP-1 activity. PARP-1 activation could be involved in the pathophysiological process of DCM by promoting oxidative stress, the inflammatory response, apoptosis and myocardial fibrosis[4-6].

To date, it has been confirmed that glucagon-like peptide-1 (GLP-1) can reduce the apoptosis of islet microcirculation endothelial cells and protect islet tissue through the PARP-1/iNOs pathway[7], but whether GLP-1 can protect cardiomyocytes by inhibiting the expression of PARP-1 is still unclear. This experiment studied different doses of the liraglutide in rats with diabetic cardiomyopathy after treatment. Blood glucose, blood lipids and pathological changes in the heart were recorded in the observation group, and an immunohistochemical method was used to observe the expression of PARP-1 in cardiac tissue to further clarify the etiology and related mechanism of liraglutide in myocardial injury

in type 2 diabetes.

MATERIALS AND METHODS

Subjects

Forty 6-week-old SPF male SD rats were purchased from the Animal Center of Shanxi Provincial People's Hospital [experimental animal license number: SYXK (Jin) 2019-0003], weighing 160-240 g, with 3-4 rats/cage. The feeding room was well ventilated, the room temperature was maintained at 18-22 °C, and the light was set in an alternating 12 h day and night cycle. The rats ate and drank freely. Animal experiments were conducted in strict accordance with the relevant regulations of experimental animal ethics.

Firstly, establishment of animal models: (1) Forty healthy male SD rats weighing 160-240 g were randomly divided into a normal control group (n = 10) and an experimental group (n = 30). They were fed an ordinary diet and a high-sugar and high-fat diet for 4 wk, respectively; (2) At the end of the fourth week of feeding, the rats in the experimental group fasted without water for 12 h overnight, and the next day, after fasting and weighing, 1% streptozotocin (STZ) was injected intraperitoneally at a dose of 40 mg/kg once, and the rats were fed a high-sugar and high-fat diet to ensure adequate drinking water and dry padding. Normal control rats were intraperitoneally injected with the same dose of normal saline. Three days after injection, if blood glucose of a random draw was more than 16.7 mmol/L and/or fasting blood glucose (FBG) was more than 11.1 mmol/L and polydipsia, polyuria, and hypereating were observed, the rats were considered type 2 diabetic rats. In the modeling process, 2 died, and 2 failed; and (3) After 4 wk of feeding with high glucose and high fat, the rats in the experimental group were randomly divided into a model group and an intervention group (further divided into a high-dose group and a low-dose group) for the follow-up experiment. Four rats died during feeding.

Secondly, intervention with drugs: The normal control group, the model group and the intervention group continued to receive the normal diet or the high-glucose and high-fat diet as before, and blood glucose level was monitored at random daily. The rats with a blood glucose level outside of the successful range of the model were excluded, and the drug intervention was started after blood glucose level had stabilized. Rats in the high-dose liraglutide group and low-dose liraglutide group were subcutaneously injected with 200 μg/kg and 100 μg/kg liraglutide, respectively, once a day for 8 wk. Rats in the normal control group and model group were subcutaneously injected with 100 μg/kg normal saline. During the intervention, the rats in the model group and the intervention group continued to be fed a high-glucose and high-fat diet, while the rats in the normal control group continued to be fed an ordinary diet.

Finally, specimen collection: (1) At the end of the 8th week of liraglutide intervention, tail venous blood was collected to measure the fasting blood glucose of rats in each group with a blood glucose meter, and the rats were weighed. Then, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g according to body weight), and the blood and heart tissues were collected; (2) Blood lipid test: The blood samples were left for half an hour and centrifuged at low speed for 15 min (4 °C, 3000 rpm), and the upper layer of serum was collected and stored in a -20 °C refrigerator. Serum was collected for the determination of serum lipids and lipoprotein-associated phospholipase A2 by an automatic multifunctional biochemical analyzer; (3) HE staining was used to observe the morphology of the myocardium: Cardiac pathological sections were prepared, and the sections were placed in xylene for 20 min for dewaxing and then placed in 100%, 95%, 85%, 75%, and 0% ethanol for 3 min each for hydration. After hydration, the sections were stained with hematoxylin for approximately 10 min, washed with water, differentiated with alcohol hydrochloric acid for a few seconds, washed with water, immersed in eosin for approximately 1 min and then washed again. Finally, the slices were dehydrated and cleared for a second time, and then the slices were sealed with glycerin gelatin. The morphological changes in the myocardium were observed under a microscope; and (4) The expression of PARP-1 in myocardial tissue was detected by immunohistochemistry: The heart wax block was sliced and heated at 60 °C for 2 h. Dewaxing and hydration were performed. For antigen repair, the sections were placed in 3% hydrogen peroxide solution for 15 min to block endogenous peroxidase activity and rinsed with phosphate buffer saline (PBS) 3 times for 2 min each time. A drop of blocking solution was added, excess liquid was removed, and the samples sat at room temperature for 20 min without washing. A 1:100 diluted primary antibody (rabbit IgG) was added by pipette, and the samples were stored at 4 °C overnight. After equilibration to room temperature for 20 min, the samples were washed 3 times with PBS (2 min each time), polymerized HRP-labeled anti-rabbit IgG was added, and the samples were incubated at 37 °C for 30 min and washed 3 times with PBS (5 min each time). Color development was followed by dehydration, transparency, sealing, and observation. The sections were observed under a light microscope, and images were collected.

Statistical analysis

One-way analysis of variance was used for comparisons between different groups, and the LSD t test

was used for pairwise comparisons within groups. $P < 0.05$ was considered statistically significant. SPSS 23.0 was used for the above statistical analysis. Origin 8.0 software was used for mapping.

RESULTS

General information
During the feeding period, the rats in the normal control group had good mental acuity and easy activity. In the model group, the overall reaction was sluggish; the rats exhibited less activity, listless spirit, dry hair, and increased food and water intake, and the bedding material was often wet and needed to be replaced daily. Compared with the model group, the state of rats in the intervention group was slightly improved, and the symptoms of polydipsia, hypereating and polyuria were slightly reduced; this improvement was more pronounced in the high-dose group. During the modeling and feeding process, the blood glucose level of 2 rats was lower than the modeling standard, so they were excluded from the group, and 6 rats died, all manifesting as hypertonia and shallow rapid breathing, and immediate blood glucose could not be measured. The cause of death may be acute complications of diabetes.

Body weight and heart weight index
After liraglutide intervention, the body weight of the rats in each group was recorded weekly, and the body weight of rats in the model group increased significantly compared with that in the normal control group. After liraglutide intervention, compared with the model group, the body weight of rats in the intervention group increased slowly or even decreased, and the body weight of rats in the high-dose group increased slower and more obviously (Figure 1A). After the rats were sacrificed at the end of the 8th week, their hearts were weighed, and compared with that in the normal control group, the heart weight index of the model group was significantly increased. After liraglutide intervention, the index was decreased in the intervention compared with that in the model group, and the decrease was more obvious in the high-dose group (Figure 2). Specific values are shown in Table 1.

Comparison of blood glucose and lipid profiles of rats in each group
After liraglutide intervention, the FBG of rats in each group was recorded weekly. Compared with the that in normal control group, the FBG of rats in the model group was significantly increased, and the FBG of rats at different doses of liraglutide intervention was decreased; the decrease was more obvious in the high-dose group (Figure 1B). Compared with those in the normal control group, the levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and lipoprotein phospholipase A2 (LP-PLA2) in the model group were significantly increased, and the differences were statistically significant ($P < 0.05$). Compared with those in the model group, the levels of FPG, TC, TG, LDL-C and LP-PLA2 in the intervention group were significantly decreased ($P < 0.05$), and the decrease was more obvious in the high-dose group but still higher than that in the normal control group. There was no significant difference in high density lipoprotein cholesterol (HDL-C) levels between groups before and after liraglutide intervention ($P > 0.05$). See Table 2 for details.

Pathological changes in myocardial tissue in each group
After HE staining, the samples were observed under a light microscope. The myocardial structure found in the rats in the normal control group was normal; the myocardial cells were closely arranged, the nuclei were clearly visible, the size was consistent, and the myocardial fibers were arranged neatly. In the model group, the myocardial fibers were disordered or even broken, the number of normal cardiomyocytes was reduced, the cells were hypertrophic, and the edges of the nuclei were unclear. The myocardial structural injury of rats in the intervention group was improved to different degrees, the myocardial fibers were arranged neatly, and the morphology of myocardial cells was normal; the improvement was more obvious in the high-dose group. Pathological results are shown in Figure 3.

Comparison of immunohistochemistry of myocardial tissue in each group
PARP-1 was expressed in the nucleus of the myocardium, and the positive expression of PARP-1 was indicated by brown-yellow particles after immunohistochemical staining. Compared with that in the normal control group, the expression of PARP-1 in the myocardium of the model group was significantly increased ($P < 0.05$). Compared with that in the model group, the expression of PARP-1 in the intervention group was significantly decreased, and the expression of PARP-1 in the myocardium of the high-dose group was more significantly decreased than that of the low-dose group ($P < 0.05$). See Table 3 and Figure 4.

Table 1 Comparison of body weight and heart weight index of rats in each group (mean ± SD)		
Group	Weight	Heart weight index
N	381 ± 22.5	2.58 ± 0.28
M	476.2 ± 27.5[a]	4.46 ± 0.31[a]
LL	451.5 ± 10.4[ab]	3.98 ± 0.26[ab]
HL	430.1 ± 13.5[abc]	3.03 ± 0.33[abc]

[a]$P < 0.05$, compared with group N.

[b]$P < 0.05$, compared with group M.

[c]$P < 0.05$, compared with group LL.

N: Normal control; M: Model; HL: High-dose liraglutide; LL: Low-dose liraglutide.

Table 2 Comparison of blood glucose and blood lipids of rats in each group (mean ± SD)						
Group	FBG	TG	TC	LDL	HDL	LP
N	5.7 ± 0.9	1.2 ± 0.28	1.73 ± 0.44	0.66 ± 0.25	1.5 ± 0.57	298 ± 41
M	25.9 ± 2.8[a]	2.84 ± 0.56[a]	4.27 ± 0.56[a]	5.49 ± 2.37[a]	1.05 ± 0.19	667 ± 79[a]
LL	20.9 ± 1.1[ab]	2.24 ± 0.52[ab]	3.32 ± 0.7[ab]	3.56 ± 0.73[ab]	1.1 ± 0.2	549 ± 45[ab]
HL	18.9 ± 1.3[abc]	1.67 ± 0.39[abc]	2.66 ± 0.76[abc]	2.06 ± 0.77[abc]	1.42 ± 0.64	448 ± 130[abc]

[a]$P < 0.05$, compared with group N.

[b]$P < 0.05$, compared with group M.

[c]$P < 0.05$, compared with group LL.

N: Normal control; M: Model; HL: High-dose liraglutide; LL: Low-dose liraglutide. FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; LP: Lipoprotein.

Table 3 Expression of polyadenosine diphosphate-ribose polymerase-1 in myocardial tissue of rats in each group (mean ± SD)		
Group	Number	PARP-1
N	8	10.92 ± 3.59
M	8	58.12 ± 5.31[a]
LL	8	42.83 ± 1.14[ab]
HL	8	23.61 ± 0.92[abc]

[a]$P < 0.05$, compared with group N.

[b]$P < 0.05$, compared with group M.

[c]$P < 0.05$, compared with group LL.

PARP-1: Polyadenosine diphosphate-ribose polymerase-1; N: Normal control; M: Model; HL: High-dose liraglutide; LL: Low-dose liraglutide.

DISCUSSION

Type 2 diabetes mellitus (T2DM) is a chronic and progressive metabolic disease that can lead to multisystem and multiorgan damage. Cardiovascular complications of diabetes mellitus are one of the main causes of death in patients with diabetes, and the trend is increasing every year. The fasting blood glucose, total cholesterol, low-density lipoprotein cholesterol, triglyceride, and heart weight index of rats in the experimental model group were significantly increased, and myocardial fiber arrangement disorder, interruption, cardiomyocyte hypertrophy, and loose shape indicated that T2DM causes myocardial injury[8,9]. Cardiac histopathological changes were consistent with T2DM and DCM, indicating successful modeling. At present, there are no specific and effective drugs for the treatment of diabetic cardiomyopathy, the progress of which can be slowed by improving lifestyle and controlling blood glucose.

DCM is a multifactorial disease that is closely related to cardiomyocyte apoptosis, oxidative stress, the inflammatory response and myocardial fibrosis. However, the exact molecular mechanism of DCM has not been thoroughly studied. Hyperglycemia can increase oxidative stress and nitrosation stress in
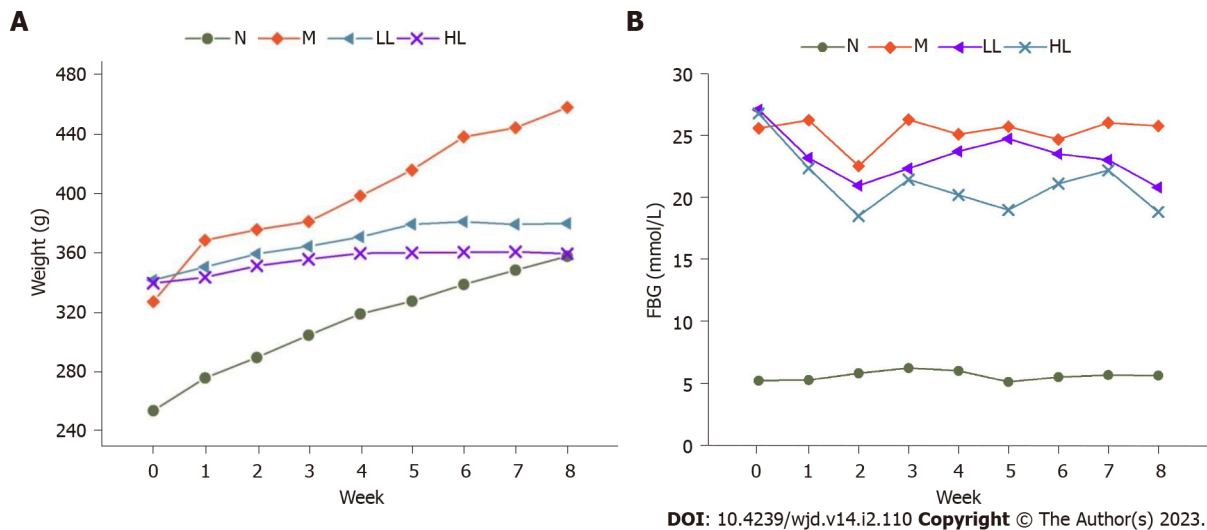
Figure 1 Weekly body weight and fasting blood glucose trend of rats in each group after liraglutide intervention. A: Weekly body weight; B: Fasting blood glucose trend. N: Normal control; M: Model; HL: High-dose liraglutide; LL: Low-dose liraglutide; FBG: Fasting blood glucose.
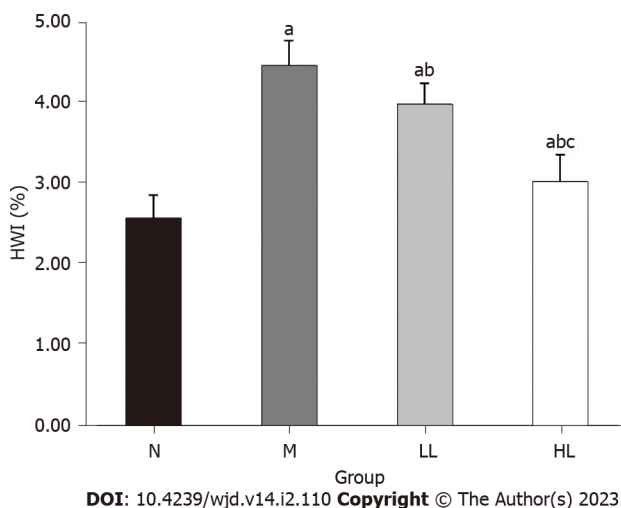
Figure 2 Comparison of heart weight index in each group. Compared with group N, [a]$P < 0.05$; compared with group M, [b]$P < 0.05$; compared with group L, [c]$P < 0.05$. HWI: Heart weight index; N: Normal control; M: Model; HL: High-dose liraglutide; LL: Low-dose liraglutide.

cells; induce DNA strand breaks; over activate PARP-1; mediate the activation of PKC, the hexosamine pathway, and the polyol pathway; activate the transcription factor NF-κB; promote the expression of genes related to the inflammatory response; and lead to apoptosis and inflammation of cardiomyocytes [10]. This causes structural and functional changes in the heart. *In vitro* experiments have shown that PARP-1 activity in cardiomyocytes in a high-glucose environment is significantly increased, and *in vivo* experiments have shown that PARP-1 gene deletion in mice in a high-glucose environment reduced cardiomyocyte apoptosis and inflammation compared with that in wild-type mice[11]. HE staining analysis of the myocardial tissues of the two groups of mice also indicated that the myocardial fibrosis of PARP-1 gene null mice was significantly improved compared with that of wild-type mice[2]. Zakaria *et al*[12] showed that PARP-1 activity in type 2 diabetic rats was significantly increased compared with that in the control group. After 10 wk of PARP-1 inhibitor (4-AB) treatment, myocardial oxidative stress and inflammation were alleviated, and myocardial fibrosis and microvascular activity were further improved. Therefore, PARP-1 plays an important role in the development of diabetic cardiomyopathy. The results of this study also showed that the expression of PARP-1 in cardiomyocytes of the model group was significantly increased compared with that of the normal control group, suggesting that PARP-1 was involved in high glucose-induced myocardial injury.

Liraglutide, as a GLP-1 agonist, can increase insulin secretion by inhibiting glucagon secretion, promoting proliferation and reducing apoptosis of isletβ-cells, thus smoothly lowering glucose. In recent years, GLP-1 receptor agonists have attracted much attention because of their extensive pharmacological effects. GLP-1 receptors are widely distributed in tissues and organs throughout the body.
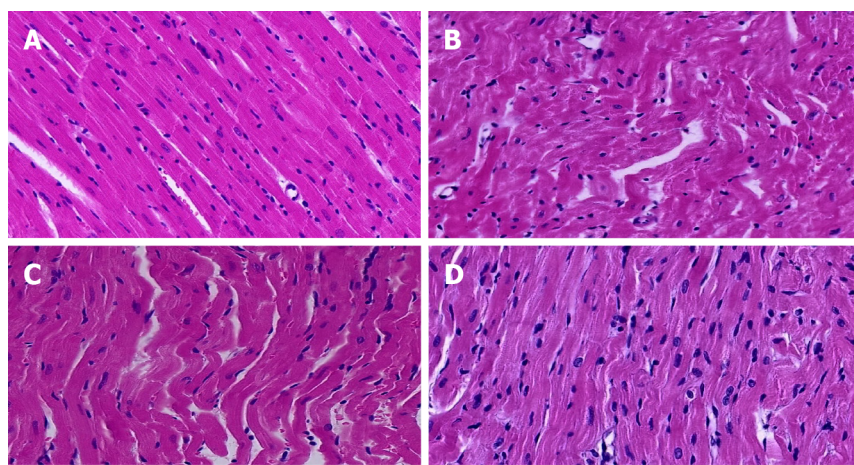
Figure 3 Haematoxylin and eosin staining of the hearts of rats in each group (400×). A: Normal control group; B: Model group; C: Low-dose liraglutide group; D: High-dose liraglutide group.
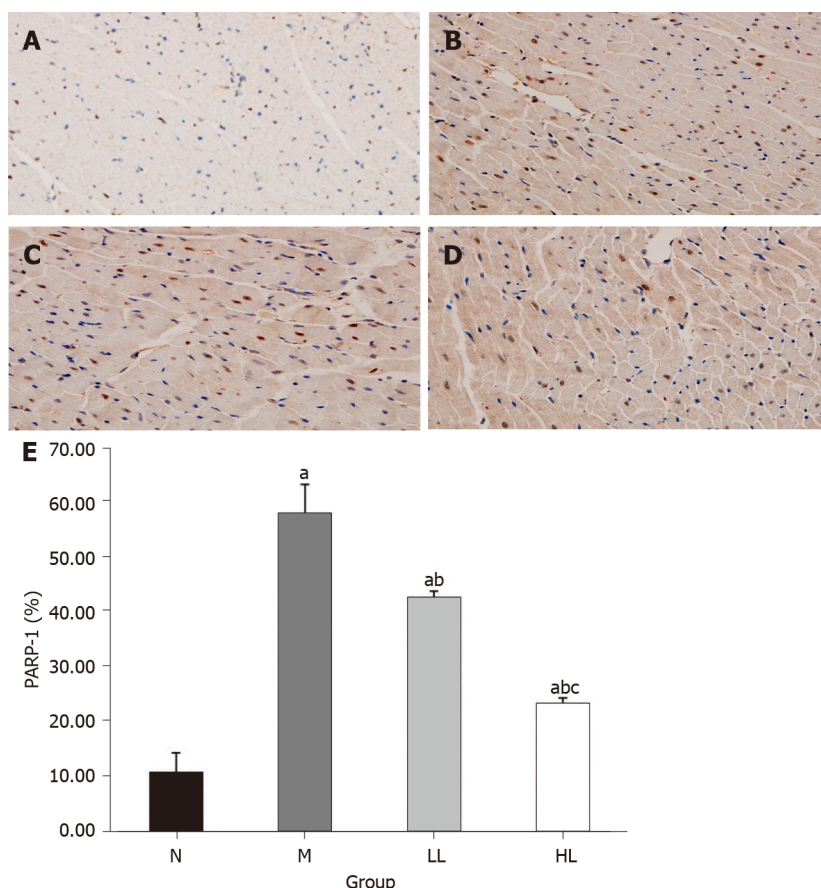
Figure 4 Comparison of immunohistochemistry of myocardial tissue in each group. A-D: Polyadenosine diphosphate-ribose polymerase-1 (PARP-1) expression in heart tissue of rats in normal control group (A), model group (B), low-dose liraglutide group (C), and high-dose liraglutide group (D); E: Comparison of PARP-1 positive expression in heart tissue of rats in each group. Compared with group N, [a]$P < 0.05$; compared with group M, [b]$P < 0.05$; compared with group L, [c]$P < 0.05$. PARP-1: Polyadenosine diphosphate-ribose polymerase-1; N: Normal control; M: Model; HL: High-dose liraglutide; LL: Low-dose liraglutide.

Studies have found that GLP-1 receptors are distributed in coronary arteries, cardiomyocytes and vascular endothelial cells, regulating and maintaining the normal physiological structure and function of the myocardium[13]. Many studies have shown that GLP-1 can reduce oxidative stress injury and apoptosis of cardiomyocytes induced by high glucose and has a protective effect on cardiomyocytes. Some researchers have shown that liraglutide, a GLP-1 receptor agonist, can reduce the inflammatory response and oxidative stress of vascular endothelial cells by inhibiting the NF-κB signaling pathway in

vascular endothelial cells and reducing the activity of NADPH oxidase. In a rat model of heart failure, GLP-1 significantly improved cardiac ejection function and the survival rate of rats after myocardial infarction compared with those in the control group. In addition, the LEADER and SUSTAIN-6 studies have confirmed that liraglutide can clinically reduce cardiovascular morbidity and mortality in patients with T2DM and high cardiovascular risk and has a comprehensive cardiovascular protective effect. The possible mechanisms include reduction of atherosclerosis, systolic blood pressure, and pulmonary capillary pressure; improvement of endothelial function; and increase of myocardial rescue rate after myocardial infarction. In conclusion, liraglutide can protect cardiomyocytes and improve cardiac function, and PARP-1 is closely related to high glucose-induced myocardial injury. Therefore, we speculated that liraglutide could inhibit the expression of cardiac PARP-1 and thereby delay the progression of DCM. In this experiment, we found that after liraglutide intervention, the myocardial injury of rats in the intervention group was significantly reduced compared with that in the model group, and the expression of PARP-1 was significantly reduced, suggesting that the protective effect of liraglutide on cardiomyocytes was related to the reduction of PARP-1 activity. In animal experiments, the current intelligent animal experiment method based on deep learning can obtain the adaptation degree of animals in various environments and the posture and state of animals after intervention, according to fitness of each experiment area, and posture and state of experiment body, which is more conducive to the establishment of animal models and the prediction and evaluation of the effects of drug intervention. This technology belongs to the frontier field at present. It is very helpful for the follow-up research of this experiment. In the future, large-scale in-depth research on animal intervention experiments will be carried out.

In conclusion, through the detection of heart weight index, blood lipids, and PARP-1 expression and observation of cardiac pathological changes in this experiment, we found that liraglutide can delay the occurrence and development of DCM by reducing the expression of cardiac PARP-1, which provides evidence for its clinical application in DCM. However, there were still some shortcomings in this experiment. For example, the TUNEL method was not used to detect cardiomyocyte apoptosis, and there was insufficient evidence of pathological changes in cardiomyocyte morphology based only on cardiac HE staining. In addition, in recent years, a study found that excessive expression of insulin-like growth factor 1 (IGF-1) can reduce myocardial infarction and myocardial cell apoptosis but can also reduce the nonocclusive coronary artery stenosis of genetically modified mice and myocardial cell death after myocardial infarction. IGF-1r cascades the activation of the PI3K/Akt signaling pathway and promotes cell proliferation. However, due to the limited time frame of this study, whether liraglutide can activate the IGF-1/PI3K/Akt pathway by inhibiting PARP-1 to protect cardiomyocytes remains unclear, and further studies are needed.

CONCLUSION

Low-dose STZ combined with a high-glucose and high-fat diet can successfully establish a rat model of T2DM. After 4 wk of continuous feeding, myocardial injury can occur, which is consistent with DCM. Liraglutide reduced the body weight of type 2 diabetic rats and significantly improved the fasting blood glucose and lipid profile in a dose-dependent manner. Liraglutide may improve myocardial injury in type 2 diabetic rats by inhibiting the expression of myocardial PARP-1 in a dose-dependent manner.

ARTICLE HIGHLIGHTS

Research background
Glucagon-like peptide-1 (GLP-1) can reduce the apoptosis of islet microcirculation endothelial cells and protect islet tissue through the polyadenosine diphosphate-ribose polymerase-1 (PARP-1)/iNOs pathway.

Research motivation
Whether GLP-1 can protect cardiomyocytes by inhibiting the expression of PARP-1 is still unclear.

Research objectives
This study investigated the mechanism of liraglutide in improving myocardial injury in type 2 diabetic rats, further clarified the protective effect of liraglutide on the heart, and provided a new option for the treatment of diabetic cardiomyopathy (DCM).

Research methods
After successful modeling, the rats were fed a high-glucose and high-fat diet for 8 wk and then started drug intervention. Blood samples were collected from the abdominal aorta to detect fasting blood

glucose and lipid profiles. Intact heart tissue was dissected, and its weight was used to calculate the heart weight index. Hematoxylin and eosin staining was used to observe the pathological changes in the myocardium and the expression of PARP-1 in the heart by immunohistochemistry.

Research results

After liraglutide intervention, compared with the model group, the expression of PARP-1 in myocardial tissue was decreased, and the reduction was more obvious in the high-dose group ($P < 0.05$) but still higher than that in the normal control group.

Research conclusions

Liraglutide may improve myocardial injury in type 2 diabetic rats by inhibiting the expression of myocardial PARP-1 in a dose-dependent manner.

Research perspectives

Through the detection of heart weight index, blood lipids, and PARP-1 expression and observation of cardiac pathological changes in this experiment, we found that liraglutide can delay the occurrence and development of DCM by reducing the expression of cardiac PARP-1, which provides evidence for its clinical application in DCM.

FOOTNOTES

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Exposure to proton pump inhibitors and risk of diabetes: A systematic review and meta-analysis

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Abstract

BACKGROUND
Exposure to proton pump inhibitors (PPIs) has been reported to have a potential role in the development of diabetes.

AIM
To determine the association between PPIs and diabetes.

METHODS
This meta-analysis is registered on PROSPERO (CRD42022352704). In August 2022, eligible studies were identified through a comprehensive literature search. In this study, odds ratios were combined with 95% confidence intervals using a random-effects model. The source of heterogeneity was assessed using sensitivity analysis and subgroup analysis. The publication bias was evaluated using Egger's test and Begg's test.

RESULTS
The meta-analysis included 9 studies with a total of 867185 participants. Results showed that the use of PPIs increased the risk of diabetes (odds ratio = 1.23, 95% confidence interval: 1.05-1.43, n = 9, I^2 = 96.3%). Subgroup analysis showed that geographic location and study type had significant effects on the overall results. Both Egger's and Begg's tests showed no publication bias (P > 0.05). Sensitivity analysis also confirmed the stability of the results.

CONCLUSION
The results of this study indicated that the use of PPIs was related to an increased risk of diabetes. However, more well-designed studies are needed to verify these results in the future.

Key Words: Proton pump inhibitors; Diabetes mellitus; Odds ratio; Meta-analysis; Diabetogenesis

Core Tip: Exposure to proton pump inhibitors has been reported to have a potential role in the development of diabetes. There are no consistent results for the association between proton pump inhibitors use and diabetes risk. This meta-analysis aimed to provide a more reliable assessment.

INTRODUCTION

Diabetes is one of the fastest-growing chronic diseases in the 21st century and is characterized by inadequate insulin production or insulin resistance. Based on the 2021 International Diabetes Federation's Diabetes Atlas, approximately 537 million adults aged 20-79 have diabetes, which is expected to reach 783 million by 2045[1]. Drug-induced diabetes is widely reported clinically and is a global problem[2].

Proton pump inhibitors (PPIs) that act on H+/K+-ATPase and inhibit gastric acid secretion are often used in the treatment of gastroesophageal reflux disease, peptic ulcer disease, and bleeding caused by nonsteroidal anti-inflammatory drugs[3]. As one of the most widely used drugs in the world, the overuse of PPIs is increasingly prominent and may cause a variety of adverse effects, including fractures, chronic kidney disease, cancer, *etc*[3-5]. Several recent studies have shown that there is a relationship between PPIs use and diabetes risk, with the potential mechanisms including changes in gut microbiota, PPI-induced hypomagnesemia, reduction of insulin-like growth factor-1, activation of pregnane X receptor, and effects of gastrin[6].

Some studies support a link between PPI use and diabetes risk, but there have been reports of conflicting conclusions. For example, two recent case-control studies and four cohort studies confirmed that PPIs were related to an increased risk of diabetes[6-10]. By contrast, PPIs were related to a reduced risk of diabetes in another cohort study[11]. However, there was no relationship between PPI use and diabetes risk in one randomized controlled trial and one cohort study[10,12]. Moreover, a recent meta-analysis involving eight studies from six articles showed that the use of PPIs was not related to the risk of diabetes[13]. Given the high prevalence of diabetes, the widespread use of PPIs, and conflicting findings about the association between PPI use and diabetes risk, this meta-analysis aimed to provide more reliable evidence on the relationship between PPI use and diabetes risk.

MATERIALS AND METHODS

Protocol and registration

This meta-analysis was conducted according to the standard Preferred Reporting Items for Systematic Review and Meta-Analysis[14]. The study protocol has been registered on the PROSPERO International Prospective Register for Systematic Review (CRD42022352704), which provides more details.

Search strategy

A comprehensive search was performed in Web of Science, PubMed, Cochrane, and Embase to collect all eligible studies published before August 2022 (Supplementary Tables 1-4). The following retrieval strategy was used: Diabetes Mellitus, DM, T2DM, Diabetes, Type 1 Diabetes or Type 2 Diabetes, and Proton Pump Inhibitor, Proton Pump Inhibitors, PPI, PPIs, Esomeprazole, Rabeprazole, Pantoprazole, and Lansoprazole or Omeprazole.

Study selection

The following studies were considered to be eligible for inclusion: (1) Randomized controlled trial, cohort study, or case-control study; (2) PPI use as an exposure of interest (no limitation on the type of PPIs); (3) Studies showing the association between PPI use and diabetes risk; and (4) Studies with relative risks, odds ratios (ORs), or hazard ratios with corresponding 95% confidence intervals (CIs). The exclusion criteria included: (1) The subject of the study was not human; (2) Reviews, systematic reviews, meta-analyses, comments, reports, letters, guides, conference abstracts, books, *etc*; (3) Results of interest not provided; and (4) Data could not be extracted or calculated.
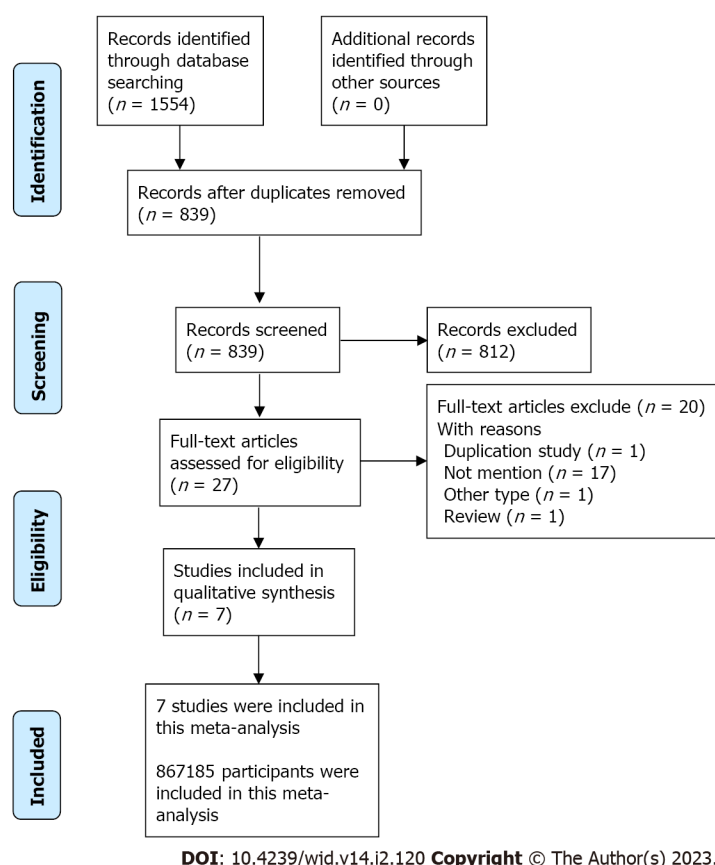
Figure 1 Flow diagram of the literature search.

Data extraction and quality assessment

Two authors separately and independently extracted data using a predesigned Excel spreadsheet. Any disagreements were resolved through discussion with the remaining authors. From the included articles, the following data were obtained: Authors' names, year of publication, country, study type, comparison, total population, age, sex, adjustment factors, and effect sizes [effect quantities (ESs), including hazard ratios or ORs] with corresponding 95%CIs.

The randomized controlled trial was assessed using the Jadad scale based on randomization and blinding and whether to describe the details of participants' exits or withdrawals from the study. The study quality was graded as follows: Low quality = 1-3 and high quality = 4-7. The Newcastle-Ottawa Scale was used to assess the quality of observational studies, including three aspects: selection, comparability, and outcome/exposure. The study quality was graded as follows: low quality = 0-3, medium quality = 4-6, and high quality = 7-9.

Statistical analysis

The data were analyzed using Stata version 15.0. (Stata Corporation, College Station, TX, United States). Due to the potential clinical heterogeneity of the studies included, multivariate-adjusted ORs were combined with 95%CIs using a random-effects model. The I^2 statistics were used to evaluate the heterogeneity between studies, and significant heterogeneity was expressed as $I^2 > 50\%$, $P < 0.1$. The subgroup analysis was conducted to further address heterogeneity. The following three aspects were considered: (1) Sex; (2) Geographic location; and (3) Study type. The sensitivity analysis was carried out by eliminating one article at a time and recalculating aggregated effect values. Egger's and Begg's tests were used to assess the publication bias, and statistical significance was expressed as the two-sided P value < 0.05.

RESULTS

Literature search and study characteristics

A total of 1554 articles were included after a comprehensive search of the four databases. After removing duplication and filtering by title and abstract, 27 articles required full-text evaluation. As shown in Figure 1, this meta-analysis contained nine studies from seven articles published from 2016 to

Table 1 Characteristics of the studies included

Ref.	Country	Study design	Quality	Population	Age in yr	Sex	Comparison	Adjustment	ESs (95%CI)
Ciardullo *et al* [7], 2022	Italian	Case-control	High	101070	≥ 40	M/F	PPIs *vs* non-PPIs	Adjusted[1]	1.56 (1.49, 1.64)
Kuo *et al*[8], 2022	China	Case-control	High	41880	55.85 ± 13.48	M/F	PPIs *vs* non-PPIs	Adjusted[2]	1.34 (1.23, 1.46)
Czarniak *et al* [6], 2022	Netherlands	Cohort	High	9531	≥ 45	M/F	PPIs *vs* non-PPIs	Adjusted[3]	1.49 (1.14, 1.95)
He *et al*[9], 2021	United Kingdom	Cohort	Moderate	470265	56.34 ± 8.11	M/F	PPIs *vs* non-PPIs	Adjusted[4]	1.56 (1.46, 1.66)
Yuan *et al* [10], 2021	United States	Cohort	High	80500	30-55	F	PPIs *vs* non-PPIs	Adjusted[5]	1.22 (1.12, 1.33)
Yuan *et al* [10], 2021	United States	Cohort	High	95550	25-42	F	PPIs *vs* non-PPIs	Adjusted[5]	1.27 (1.17, 1.38)
Yuan *et al* [10], 2021	United States	Cohort	High	28639	40-75	M	PPIs *vs* non-PPIs	Adjusted[5]	1.12 (0.91, 1.38)
Moayyedi *et al*[12], 2019	Mixed	Randomized controlled trial	High	17598	67.7 ± 8.1	M/F	PPIs *vs* placebo	Unadjusted	0.96 (0.85, 1.09)
Lin *et al*[11], 2016	China	Cohort	High	22152	55.38 ± 16.95	M/F	PPIs *vs* non-PPIs	Adjusted[6]	0.80 (0.73, 0.88)

[1]Adjusted for sex, age, and clinical status.

[2]Adjusted for age, sex, residence income, indications of proton pump inhibitor (PPI) use, obesity, dyslipidemia, hypertension, and alcohol use disorders.

[3]Adjusted for age, sex, PPI past use, body mass index (BMI), hypertension, current smoking, alcohol consumption, physical activity, and education levels.

[4]Adjusted for sex, with additional adjustment for diabetes risk factors, including age at recruitment, ethnicity, deprivation, BMI, smoking status, family history of diabetes in a first-degree relative, cardiovascular disease, treated hypertension, corticosteroids use, diagnosis of schizophrenia or bipolar affective disorder, learning disabilities, diagnosis of gestational diabetes, diagnosis of polycystic ovary syndrome, atypical antipsychotics, statins and clinical indications for PPI use.

[5]Adjusted for race, family history of diabetes, BMI, number of pack-years of smoking, alcohol intake per day, physical activity, overall diet quality, total calorie intake, multivitamin use, history of hypertension, hypercholesterolemia, cancer, menopausal status and postmenopausal hormone use in women, number of parity in women, breastfeeding in women, any use of antibiotics, regular non-steroidal anti-inflammatory drug use, any use of steroids, gastroesophageal reflux disease, gastric or duodenal ulcer, upper gastrointestinal tract bleeding, and regular use of H2 receptor agonists.

[6]Adjusted for age, sex, hypertension, gout and/or hyperuricemia, coronary artery disease, stroke, pancreatitis, hyperlipidemia, obesity, H2 blocker use, and clozapine or olanzapine use.

CI: Confidence interval; ESs: Effect quantities; F: Female; M: Male; PPI: Proton pump inhibitor.

2022, including one randomized controlled trial, two case-control studies, and six cohort studies, which involved 867185 participants. Table 1 shows the baseline characteristics and quality assessments of the studies included.

Meta-analysis

Meta-analysis using a random-effects model indicated a statistically significant association between PPI use and diabetes risk compared to placebo or no PPI use (OR = 1.23, 95%CI: 1.05-1.43, *n* = 9, I^2 = 96.3 %) (Figure 2A).

Subgroup analysis

The subgroup analysis was conducted based on sex, geographic location, and study type since the I^2 analysis revealed significant heterogeneity: (1) Sex. The use of PPIs was related to an increased risk of diabetes in both females (pooled ES = 1.37, 95%CI: 1.17-1.59, *n* = 4, I^2 = 91.8%) and males (pooled ES = 1.34, 95%CI: 1.17-1.55, *n* = 3, I^2 = 78.2%) (Figure 2B); (2) Geographic location. A statistical association was found in Europe (pooled ES = 1.56, 95%CI: 1.50-1.62, *n* = 3, I^2 = 0%) and North America (pooled ES = 1.24, 95%CI: 1.17-1.31, *n* = 3, I^2 = 0%) but not in Asia (pooled ES = 1.04, 95%CI: 0.62-1.72, *n* = 2, I^2 = 98.4%) (Figure 2C); and (3) Study type. A statistical association was detected in case-control studies (pooled ES = 1.45, 95%CI: 1.25-1.68, *n* = 2, I^2 = 89.1%) but not in cohort studies (pooled ES = 1.21, 95%CI: 0.98-1.50, *n* = 6, I^2 = 96.3%) (Figure 2D).

Sensitivity analysis

The sensitivity analysis was performed by excluding one study at a time and recalculating pooled risk estimates. The results showed no significant change in risk estimates after combination (Figure 3A). This analysis verified the robustness of the results of this study.
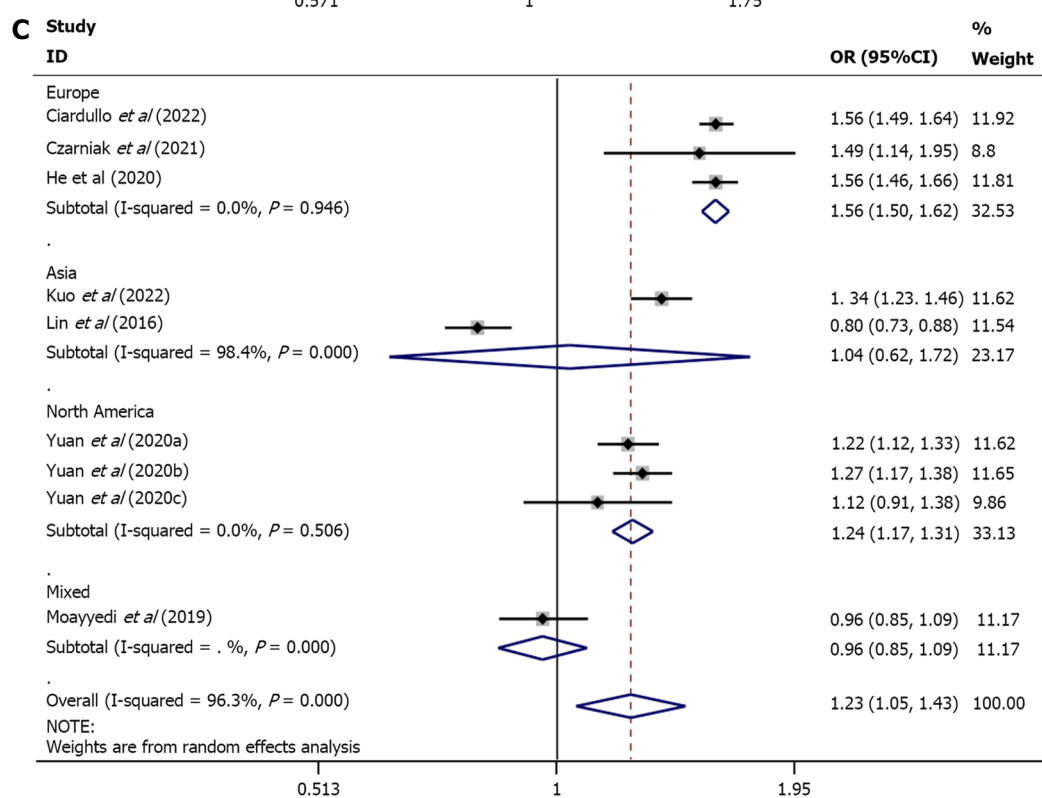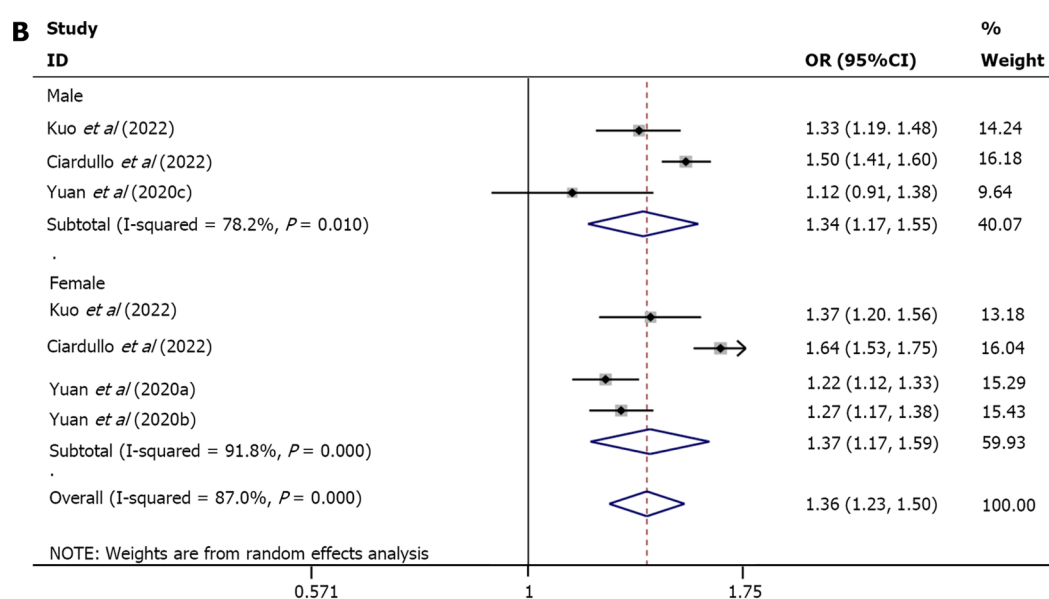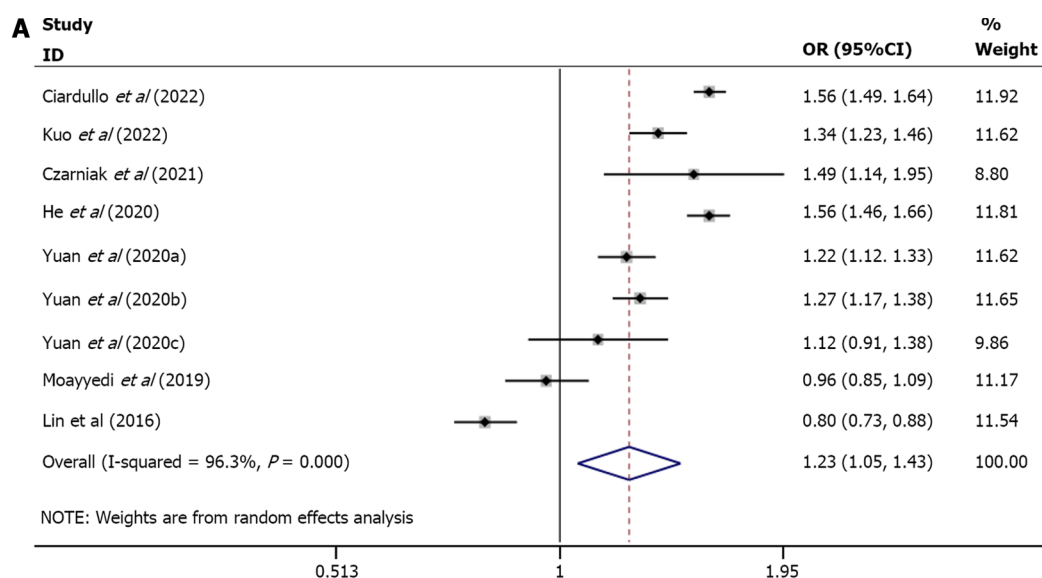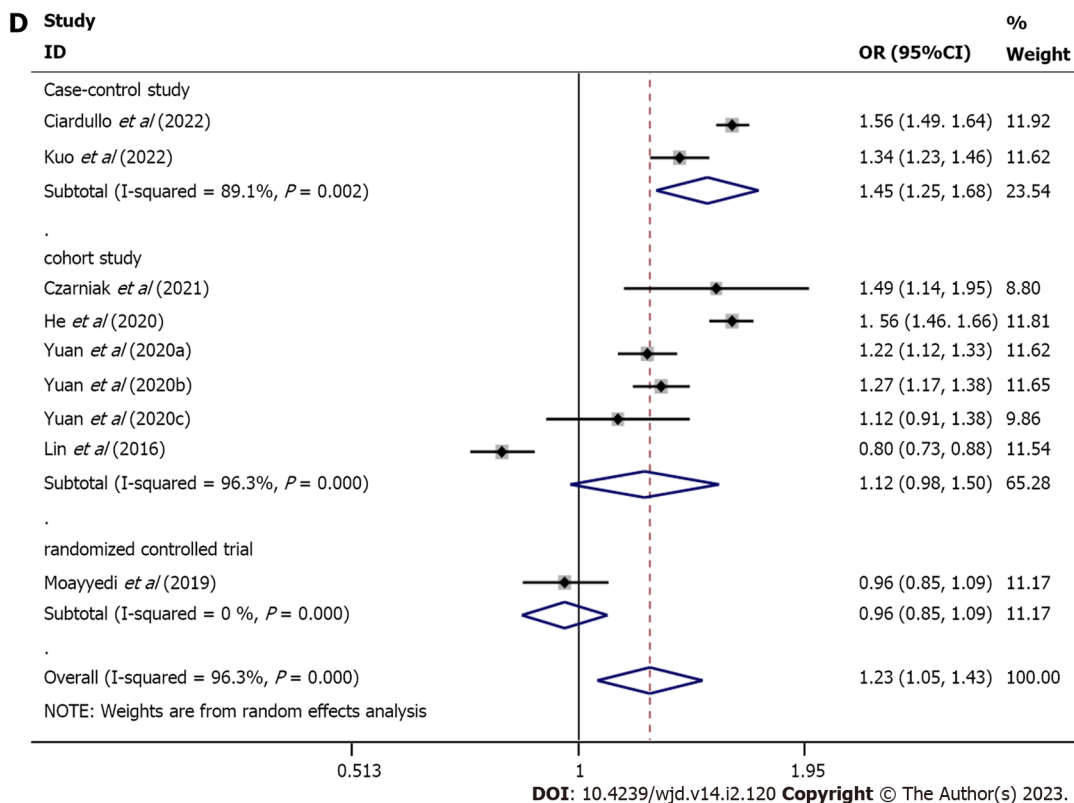
Figure 2 Forest plots. A: Forest plot of the association between proton pump inhibitor use and diabetes risk; B: Forest plot of the association between proton pump inhibitor use and diabetes risk according to sex; C: Forest plot of the association between proton pump inhibitor use and diabetes risk according to geographic location; D: Forest plot of the association between proton pump inhibitor use and diabetes risk according to study design. CI: Confidence interval; OR: Odds ratio.

Publication bias

Egger's test (P = 0.149) and Begg's test (P = 0.175) indicated no publication bias (Figure 3B and C).

DISCUSSION

The meta-analysis, including nine studies with a total of 867185 participants, showed that the use of PPIs increased the risk of diabetes, which is consistent with some previous studies. A case-control study of 41880 participants in China and a cohort study of 9531 participants in Europe, after adjusting for known risk factors, showed that the use of PPIs was related to an increased risk of type 2 diabetes, and efficacy was dose-dependent[6,8]. However, the former showed an increased risk of type 2 diabetes in patients treated with pantoprazole, lansoprazole, and omeprazole, while no increased risk was found in patients treated with esomeprazole or rabeprazole[8]. Another case-control study from Europe involving 101070 participants showed that the long-term use of PPIs was related to an increased risk of diabetes and increased risk over time with treatment[7]. Similarly, there was a positive relationship between PPI use and diabetes risk in two cohort studies in North America and one in Europe but not in another study in North America[9,10].

In contrast, a randomized controlled trial of 17598 participants from mixed regions found no statistical difference between pantoprazole and diabetes risk, without adjusting for confounding factors [12]. Moreover, a cohort study from China, including 22152 participants, showed that the use of PPIs was related to a reduced risk of diabetes[11]. Recently, a systematic review and meta-analysis summarized the evidence on this topic, including eight studies of 850019 participants, showing that the use of PPIs was not related to an increased or decreased risk of diabetes. However, PPIs were only used as controls in two unadjusted included studies, with glucocorticoids and antipsychotics that have been reported to have a risk of diabetes in experimental groups[15-18]. The criteria for the included studies were standardized, and three recent high-quality studies were supplemented in the meta-analysis[6-8].

The mechanism between PPI use and diabetes risk is still unclear, and several hypotheses exist. First, the use of PPIs can alter the gut microbiome[19,20]. Changes in the gut microbiome environment play an important role in metabolism that are related to obesity, metabolic syndrome, insulin resistance, and the development of diabetic microvascular and macrovascular complications[21,22].
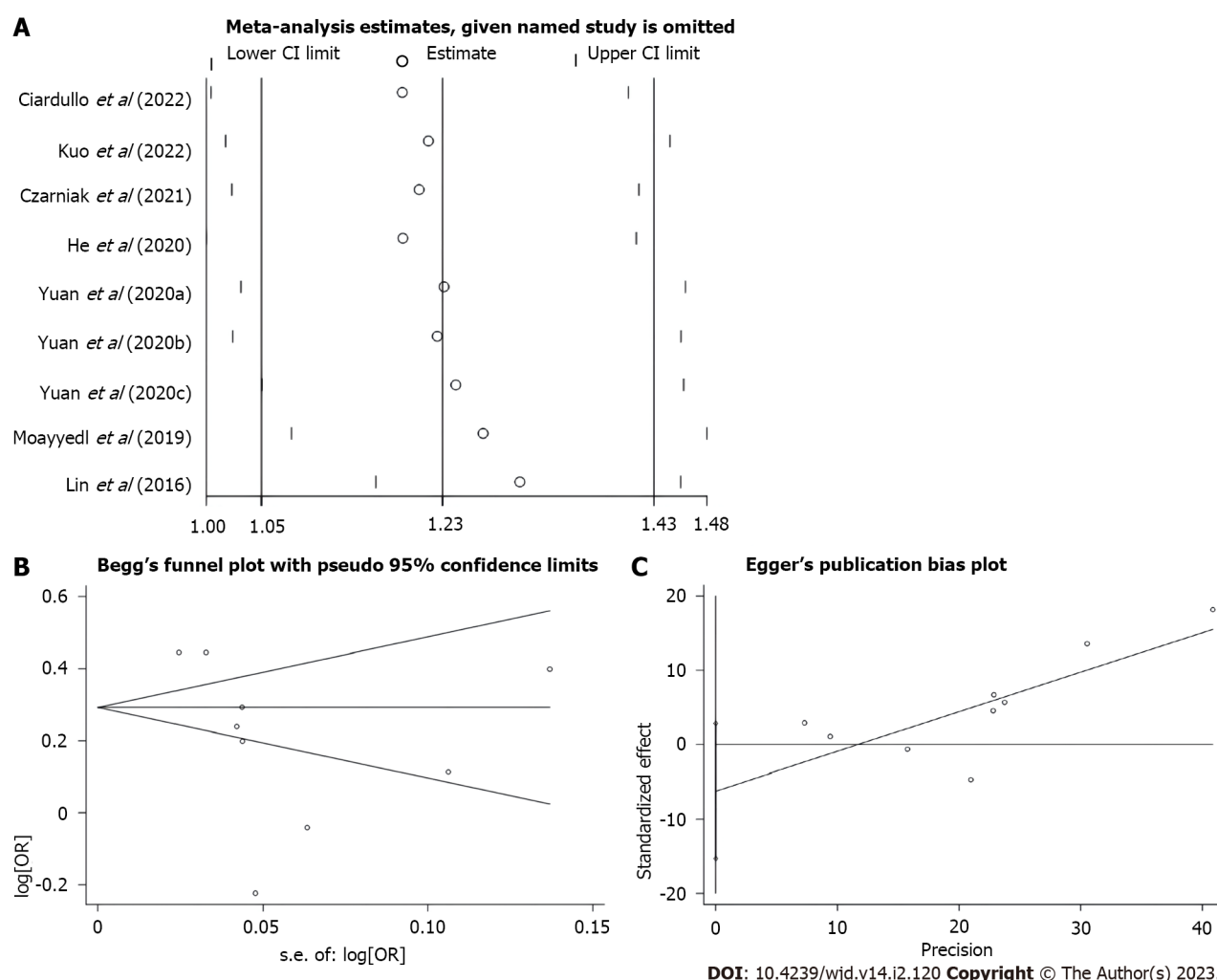
Figure 3 Plots. A: Plot of sensitivity analysis by excluding one study each time and pooling estimate for the remaining studies; B: Plot of Begg's test; C: Plot of Egger's test. CI: Confidence interval; OR: Odds ratio.

Second, studies have shown that the use of PPIs can lead to hypomagnesemia[23,24]. Magnesium, an essential mineral in the human body and the second richest cationic ion in cells, activates enzymes and plays an important role as a cofactor in various biochemical reactions[25]. It has been also reported that lower serum magnesium concentrations are associated with higher insulin resistance and diabetes risk, with a nonlinear dose-response relationship[26,27].

Third, studies have shown a negative correlation between PPIs and insulin-like growth factor-1 (IGF-1) levels[28]. IGF-1 is a polypeptide protein substance that is similar to insulin in molecular structure. It is able to enhance the absorption of glucose and amino acids, promote glycogen synthesis and lactate secretion, inhibit glycogenolysis, and increase insulin sensitivity. Studies have found that low IGF-1 levels are associated with diabetes risk[29,30].

The fourth mechanism may be associated with the use of PPIs to activate progesterone X receptor (PXR)[6]. PXR is a ligand-dependent member of the nuclear receptor family that regulates target genes and senses the chemical environment, which is activated by many clinically used drugs and environmental pollutants. When activated, it can regulate the expression of multiple drug metabolizing enzymes and transporters[31]. The true mechanism by which PXR impairs glucose metabolism is not fully understood, but its role in inducing hyperglycemia/diabetes by impairing glucose metabolism in the liver has been demonstrated[32].

The fifth mechanism is considered to be associated with gastrin. PPIs have been shown to increase endogenous gastrin levels in both animals and humans by inhibiting gastric acid secretion, which is associated with islet growth/regeneration[33]. Interestingly, there is also conflicting evidence regarding the role of PPIs in glycemic control for patients with diabetes[34,35]. Perhaps gastrin may be depleted over time, increasing the risk of diabetes[7].

It is well known that heterogeneity and study quality may influence the relationship resulting from the final analysis. Due to the high degree of heterogeneity in this study, a subgroup analysis was performed to understand the origin of heterogeneity. In the geographic location subgroup, a positive association between PPI use and diabetes risk was observed in Europe and North America but not in

Asia or other mixed regions. In addition, an association between PPI use and diabetes risk was also found in the case-control study but not in the cohort study. This suggests that more high-quality studies from diverse geographic locations may be required in the future. Adjusting for confounding factors has an important influence on the reliability of meta-analysis results, and only one randomized controlled trial of the included studies was not adjusted. Sensitivity analysis showed robust results in the present meta-analysis, and there was no published bias in this study. Taken together, the results of this meta-analysis are robust.

In summary, PPI use is associated with diabetes risk. It is expected that the inclusion of more high-quality studies with detailed data on the use of PPIs can be required in the future, such as different types of PPIs, frequency of use, duration of use, and indications. H_2 receptor antagonists could also be included in the analysis to see if there is an association between antacids and diabetes.

CONCLUSION

Based on the available evidence, it can be concluded that the use of PPIs is related to an increased risk of diabetes. In addition, this connection between the use of PPIs and the risk of diabetes is also found in both females and males. However, accounting for the limitations and the presence of bias in the primary studies, future research should still focus on the use of different types of PPIs and the risk of diabetes, especially in people with different backgrounds.

ARTICLE HIGHLIGHTS

Research background
There is a still controversial connection between the widespread use of proton pump inhibitors (PPIs) and the risk of diabetes.

Research motivation
In a previous meta-analysis, the use of PPIs was shown to not be associated with the risk of diabetes. However, three recent high-quality studies found that the use of PPIs was associated with an increased risk of diabetes. Therefore, a meta-analysis was carried out to determine the association between PPIs and diabetes.

Research objectives
To provide more reliable evidence on the relationship between PPI use and diabetes risk.

Research methods
Meta-analysis was used to realize the objectives. Statistical analyses were performed by using Stata version 15.0.

Research results
Results showed that the use of PPIs increased the risk of diabetes (odds ratio = 1.23, 95% confidence interval: 1.05-1.43, $n = 9$, $I^2 = 96.3\%$). In the subgroup analysis, geographic location and study type had significant effects on the overall results. No publication bias ($P > 0.05$) was found in Egger's or Begg's tests. Also, sensitivity analysis confirmed the stability of the results.

Research conclusions
The results of this study indicated that the use of PPIs was associated with an increased risk of diabetes.

Research perspectives
It is expected that more research from diverse geographic locations with detailed data on the use of PPIs is required in the future.

FOOTNOTES

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