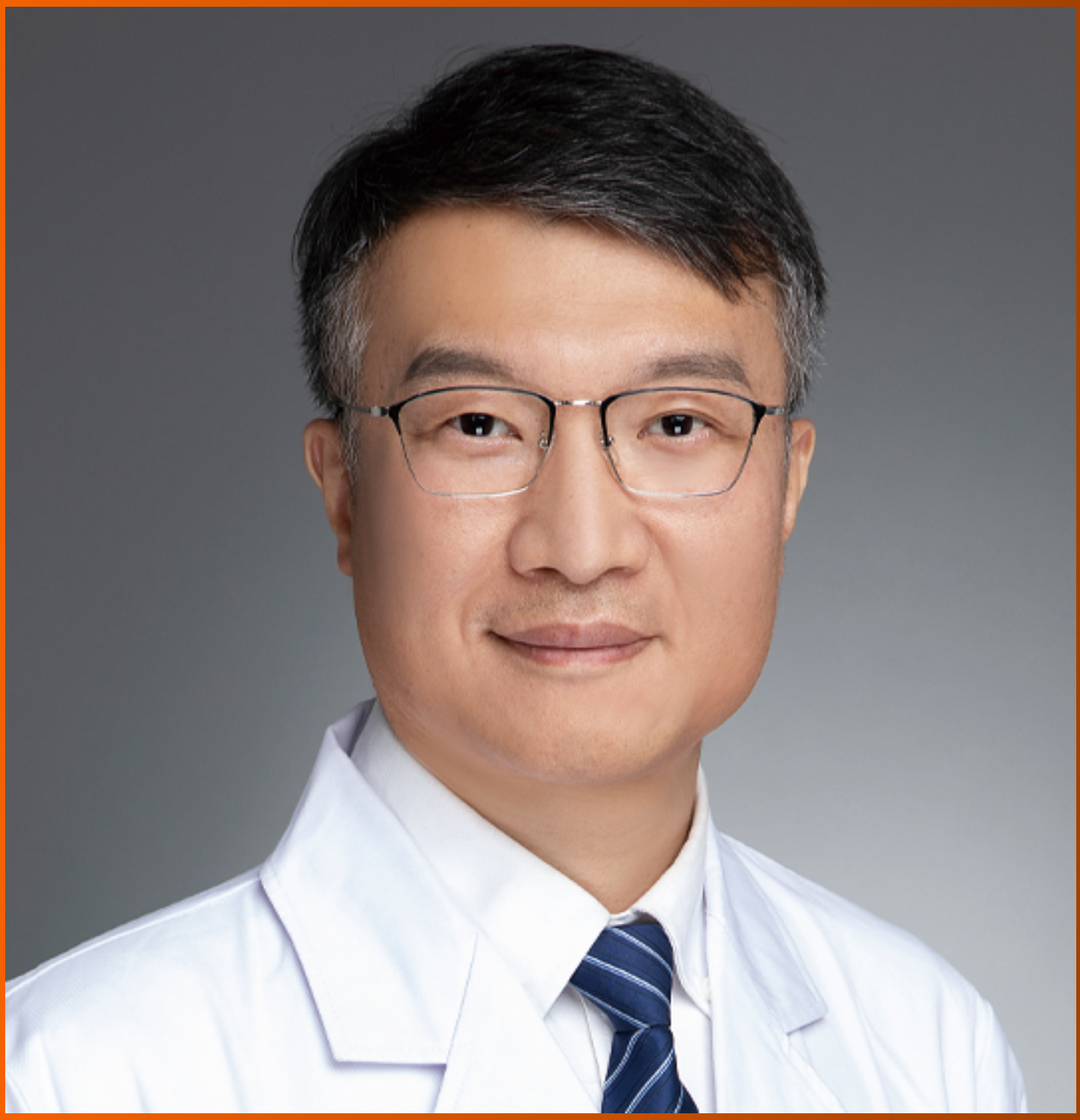
ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2021 July 15; 12(7): 916-1140



Published by Baishideng Publishing Group Inc

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RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Li-Li Wang*; **Production Department Director:** *Yu-Jie Ma*; **Editorial Office Director:** *Jia-Ping Yan*.

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Timothy Koch

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-9358/editorialboard.htm

PUBLICATION DATE
July 15, 2021

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World J Diabetes 2021 July 15; 12(7): 916-931

DOI: 10.4239/wjd.v12.i7.916

ISSN 1948-9358 (online)

FIELD OF VISION

Long-term metformin therapy and vitamin B12 deficiency: An association to bear in mind

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Author contributions: Infante M conceived and wrote the manuscript; Leoni M contributed to the literature search; Caprio M and Fabbri A revised the final version of the manuscript.

Conflict-of-interest statement: The authors declare that they have no competing interests.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

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Abstract

To date, metformin remains the first-line oral glucose-lowering drug used for the treatment of type 2 diabetes thanks to its well-established long-term safety and efficacy profile. Indeed, metformin is the most widely used oral insulin-sensitizing agent, being prescribed to more than 100 million people worldwide, including patients with prediabetes, insulin resistance, and polycystic ovary syndrome. However, over the last decades several observational studies and meta-analyses have reported a significant association between long-term metformin therapy and an increased prevalence of vitamin B12 deficiency. Of note, evidence suggests that long-term and high-dose metformin therapy impairs vitamin B12 status. Vitamin B12 (also referred to as cobalamin) is a water-soluble vitamin that is mainly obtained from animal-sourced foods. At the cellular level, vitamin B12 acts as a cofactor for enzymes that play a critical role in DNA synthesis and neuroprotection. Thus, vitamin B12 deficiency can lead to a number of clinical consequences that include hematologic abnormalities (*e.g.*, megaloblastic anemia and formation of hypersegmented neutrophils), progressive axonal demyelination and peripheral neuropathy. Nevertheless, no definite guidelines are currently available for vitamin B12 deficiency screening in patients on metformin therapy, and vitamin B12 deficiency remains frequently unrecognized

Country/Territory of origin: Italy

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 29, 2021
Peer-review started: January 29, 2021
First decision: March 16, 2021
Revised: March 21, 2021
Accepted: April 29, 2021
Article in press: April 29, 2021
Published online: July 15, 2021

P-Reviewer: Wu WJ, Xu J
S-Editor: Gao CC
L-Editor: Filipodia
P-Editor: Ma YJ

in such individuals. Therefore, in this "field of vision" article we propose a list of criteria for a cost-effective vitamin B12 deficiency screening in metformin-treated patients, which could serve as a practical guide for identifying individuals at high risk for this condition. Moreover, we discuss additional relevant topics related to this field, including: (1) The lack of consensus about the exact definition of vitamin B12 deficiency; (2) The definition of reliable biomarkers of vitamin B12 status; (3) Causes of vitamin B12 deficiency other than metformin therapy that should be identified promptly in metformin-treated patients for a proper differential diagnosis; and (4) Potential pathophysiological mechanisms underlying metformin-induced vitamin B12 deficiency. Finally, we briefly review basic concepts related to vitamin B12 supplementation for the treatment of vitamin B12 deficiency, particularly when this condition is induced by metformin.

Key Words: Metformin; Vitamin B12 deficiency; Metformin-induced cobalamin deficiency; Diabetes; Type 2 diabetes; Prediabetes; Screening criteria; Neuropathy; Anemia

Core Tip: Over the last decades, vitamin B12 deficiency has been increasingly recognized as a possible consequence of long-term metformin therapy, potentially resulting in clinical manifestations such as hematologic abnormalities and peripheral neuropathy. Metformin-induced vitamin B12 deficiency has relevant implications in light of the growing population of individuals on metformin therapy for the treatment of type 2 diabetes, prediabetes, insulin resistance and polycystic ovary syndrome on a global scale. Notwithstanding, no definite guidelines are currently available for vitamin B12 deficiency screening in metformin-treated patients. We therefore propose a list of criteria for cost-effective vitamin B12 deficiency screening in metformin-treated patients.

Citation: Infante M, Leoni M, Caprio M, Fabbri A. Long-term metformin therapy and vitamin B12 deficiency: An association to bear in mind. *World J Diabetes* 2021; 12(7): 916-931
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/916.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.916

INTRODUCTION

Vitamin B12, also known as cobalamin, is a water-soluble vitamin that is primarily obtained from animal-sourced foods such as red meat, poultry, shellfish, milk, eggs and other dairy products, or vitamin B12-fortified foods[1]. Once ingested, vitamin B12 is released from its food carrier proteins by proteolysis in the acidic environment of the stomach, where it binds to a glycoprotein called haptocorrin (also referred to as R-factor or R-protein). Haptocorrin is produced and secreted by the salivary glands. The haptocorrin-vitamin B12 complex protects vitamin B12 from degradation in the acidic environment of the stomach. Once the haptocorrin-vitamin B12 complex reaches the duodenum, pH change and degradation of haptocorrin by pancreatic proteases favor vitamin B12 cleavage from haptocorrin, resulting in the release of vitamin B12 in its free form. In the duodenum, the free vitamin B12 binds to intrinsic factor (IF), a glycoprotein secreted by gastric parietal cells, resulting in the formation of an IF-vitamin B12 complex. The newly formed IF-vitamin B12 complex subsequently binds, in a calcium-dependent manner, to the cubilin receptor (a protein encoded by the *CUBN* gene) on the enterocytes of the distal ileum, resulting in the absorption of vitamin B12 by receptor-mediated endocytosis. Upon internalization, the IF-vitamin B12 complex is released from its receptor, IF is degraded in lysosomes, and vitamin B12 enters the circulation *via* the multidrug resistance protein 1 (MDR1) transporter [2]. In the circulation, approximately 20%-25% of vitamin B12 is bound to its binding protein transcobalamin. The transcobalamin-vitamin B12 complex is also known as holotranscobalamin (HoloTC), which represents the biologically active form of cobalamin and allows for cellular uptake of vitamin B12 through specific cell surface

transcobalamin receptors[3]. The remaining 75%-80% of vitamin B12 is bound to haptocorrin and is stored in the liver. Some vitamin B12 is excreted in bile and undergoes enterohepatic circulation[1,4,5].

At the cellular level, vitamin B12 serves as a cofactor for the enzyme methionine synthase, which catalyzes the conversion of homocysteine into methionine. The overall reaction takes place in the cytosol and transforms 5-methyl-tetrahydrofolate (5-methyl-THF) into THF while transferring a methyl group to homocysteine to synthesize methionine. THF is then converted into intermediates that are used in the synthesis of pyrimidine bases of DNA. Therefore, vitamin B12 deficiency leads to homocysteine accumulation, impaired DNA synthesis, and hematologic abnormalities such as formation of hypersegmented neutrophils and megaloblastic anemia, a condition in which the bone marrow produces unusually large, structurally abnormal and immature red blood cells called megaloblasts as a consequence of ineffective hematopoiesis[4]. Anemia can be associated with various symptoms, including pallor, palpitations, tachycardia, and fatigue, which are frequently observed in patients with vitamin B12 deficiency. Although megaloblastic anemia is the most common hematologic abnormality, vitamin B12 deficiency can potentially affect all bone marrow cell lineages, resulting in pancytopenia[6].

Vitamin B12 also acts as a cofactor for the enzyme methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA, a reaction that takes place in the mitochondria[1]. Thus, vitamin B12 deficiency results in the accumulation of methylmalonyl-CoA that is subsequently converted to methylmalonic acid (MMA), whose plasma levels are often elevated in patients with vitamin B12 deficiency. In subjects with vitamin B12 deficiency, increased levels of MMA and homocysteine have been suggested to contribute to myelin damage (myelopathy) and, as a consequence, to peripheral and autonomic neuropathy[1,4,7]. Neurologic manifestations of vitamin B12 deficiency include progressive axonal demyelination, impaired sensory and peripheral nerve function, subacute combined degeneration of the spinal cord, areflexia and loss of proprioception and vibration sensitivity[5,8,9]. The aforementioned neurologic manifestations can be erroneously interpreted as features of diabetic neuropathy in diabetic patients who are chronically treated with metformin. Failure to identify the cause of neuropathy can lead to progression of central and/or peripheral nerve damage, which may be arrested, but not completely reversed, by vitamin B12 replacement in some instances[10]. Neurocognitive manifestations such as poor memory performance, cognitive impairment, dementia, delirium, depression and episodes of psychosis are also possible in the presence of severe and chronic vitamin B12 deficiency[5,8,11]. Other symptoms that have been reported in adult patients with vitamin B12 deficiency include glossitis, skin hyperpigmentation, infertility, hearing loss, bone disease and macular degeneration[8].

BIOMARKERS OF VITAMIN B12 STATUS AND DEFINITION OF VITAMIN B12 DEFICIENCY

To date, there is no consensus about the exact definition of vitamin B12 deficiency[1]. Indeed, there is still a significant debate within the scientific community about the specific cut-off values that should be applied to define a low vitamin B12 status and about the definition of the best biomarker or combination of biomarkers to assess vitamin B12 status[1,12]. Varying cut-off values invariably lead to underestimating or overestimating the incidence of vitamin B12 deficiency. With regard to the definition of an optimal vitamin B12 status, a low vitamin B12 status (frank vitamin B12 deficiency) is generally defined as total serum vitamin B12 levels of < 148 pmol/L, with levels between 148 and 221 pmol/L being considered as "borderline" or suggestive of "marginal deficiency"[5].

As a matter of fact, there has been a debate about the clinical significance of biochemical vitamin B12 deficiency *vs* true tissue deficiency, and whether subclinical (*i.e.* mild and asymptomatic) vitamin B12 deficiency represents a public health concern [13]. Discussion of issues regarding the sensitivity and specificity of individual biomarkers of vitamin B12 status led a roundtable on NHANES monitoring of those biomarkers to agree that a comprehensive and accurate assessment of true tissue vitamin B12 deficiency should include at least one biomarker of circulating vitamin B12 (total vitamin B12 or HoloTC) coupled with one functional (metabolic) biomarker of vitamin B12 status, such as MMA or total homocysteine[13]. In fact, several studies have established that serum vitamin B12 has a limited diagnostic value as a stand-alone marker because of its low specificity and sensitivity in identifying a true tissue

vitamin B12 deficiency. Low serum levels of vitamin B12 thus may not necessarily represent a true tissue deficiency[5,12]. A major limitation of the measurement of total serum vitamin B12 is that it assesses total circulating vitamin B12, of which approximately 80% is bound to haptocorrin and is therefore not bioavailable for cellular uptake. Moreover, this assay does not reliably reflect the cellular vitamin B12 status [12]. Studies assessing serum and cellular vitamin B12 showed that serum vitamin B12 levels do not always reflect cellular vitamin B12 status[12,14]. For instance, patients with inborn errors of vitamin B12 metabolism can exhibit low or normal serum values of vitamin B12, while being deficient at the cellular level[12]. In addition, severe functional vitamin B12 deficiency has been documented in the presence of normal or even elevated levels of serum vitamin B12[12], given that serum vitamin B12 levels can be maintained at the expense of cobalamin tissue stores[15].

In light of the abovementioned remarks, measurement of functional biomarkers of vitamin B12 status (homocysteine and MMA) may be useful to confirm the diagnosis of true vitamin B12 deficiency, particularly in the presence of low-normal total serum vitamin B12 levels and/or clinical suspicion of vitamin B12 deficiency[1]. Therefore, total vitamin B12, its bioactive protein-bound form HoloTC, homocysteine and MMA are the preferred serum biomarkers to accurately assess vitamin B12 status[12]. However, it is worth noting that serum levels of homocysteine and MMA can be elevated even in the presence of folate deficiency, which can also be associated with macrocytic anemia and thereby confused with vitamin B12 deficiency. Thus, measurement of serum folate, MMA and homocysteine levels can help to distinguish vitamin B12 deficiency from folate deficiency. As discussed earlier, serum levels of both homocysteine and MMA are often elevated in the presence of true vitamin B12 deficiency. Conversely, homocysteine levels are elevated but MMA levels are normal in the presence of folate deficiency[4]. Yet, it is also worth reminding that both homocysteine and MMA levels can be elevated in the presence of renal impairment[1].

CAUSES OF VITAMIN B12 DEFICIENCY

Apart from long-term metformin therapy (discussed later in the text), several causes and conditions increase the risk of vitamin B12 deficiency (Table 1), as it has been reviewed elsewhere[5,8,16]. Those conditions should be identified promptly in metformin-treated patients with vitamin B12 deficiency for a proper differential diagnosis.

Populations at risk for vitamin B12 deficiency

Specific populations at risk for development of vitamin B12 deficiency include elderly individuals, pregnant women and selected ethnic and racial groups[5]. Vitamin B12 deficiency is more common in older individuals, particularly among those over 65 years of age, who have a prevalence of cobalamin deficiency of approximately 10%-15%[5,17,18]. The prevalence of vitamin B12 deficiency is even higher in the "oldest-old", with reports of approximately 23% of octogenarians and 35% of centenarians [19]. Possible causes of vitamin B12 in elderly people range from malabsorption and/or poor dietary intake to a number of age-related comorbidities and underlying conditions (Table 1).

Pregnancy can also alter maternal vitamin B12 status by facilitating the transfer of cobalamin to the fetus and infant[5]. The actual prevalence of vitamin B12 deficiency during pregnancy appears to vary across geographic regions, being reported as lower than 10% in Brazil and Canada and greater than 70% in some areas of Turkey and India[5]. Total plasma vitamin B12 levels progressively decrease during pregnancy, and the reduction is often accompanied by a moderate increase in MMA levels, suggesting a functional depletion in intracellular cobalamin status[20,21]. The pregnancy-related decline in vitamin B12 levels may result from alterations in haptocorrin-bound cobalamin[22]. Nonetheless, the assessment of vitamin B12 status as well as the evaluation of the actual prevalence of vitamin B12 deficiency during pregnancy are challenging because of the profound anatomical and physiological changes that limit the use of the established reference ranges employed for determination of cobalamin status in non-pregnant women[5,20].

The prevalence of vitamin B12 deficiency has also been reported to vary across different ethnic and racial groups, probably because of genetic factors and/or cultural and religious practices that predispose different populations to diverse levels of dietary intake of animal products, especially red meat. In a study conducted in participants of the population-based multidisciplinary Georgia Centenarian Study,

Table 1 Causes of vitamin B12 deficiency and underlying mechanisms

Conditions potentially associated with vitamin B12 deficiency	Underlying mechanisms
General malnutrition, chronic alcohol abuse, and vegan or strict vegetarian diets	Low or inadequate dietary intake of foods containing vitamin B12
Older age	Vitamin B12 malabsorption and deficiency due to inadequate dietary intake are common in the elderly
Gastric bypass, partial or complete gastrectomy, gastric reduction, bariatric surgery and chronic gastritis due to *Helicobacter pylori* infection	Impaired IF secretion
Atrophic gastritis (an autoimmune disease characterized by the presence of antibodies directed against gastric parietal cells and IF)	Immune-mediated destruction of gastric parietal cells, gastric mucosal atrophy, hypochlorhydria, decreased IF production, subsequent vitamin B12 malabsorption, vitamin B12 deficiency and pernicious anemia (a type of megaloblastic anemia)
Long-term use (≥ 12 mo) of drugs altering gastric acid secretion or gastric pH (*e.g.*, PPIs, H2RAs and antacids)	These drugs reduce the production of hydrochloric acid by gastric parietal cells; as a consequence, vitamin B12 is not adequately released from the food matrix due to insufficient hydrochloric acid and low pepsin activity
Long-term use of metformin	The underlying mechanism accounting for metformin-induced vitamin B12 deficiency is not fully understood, although it may involve one or more of the following: (1) Interference with the calcium-dependent binding of the IF-vitamin B12 complex to the cubilin receptor on enterocytes at the ileum level; (2) Interaction with the cubilin endocytic receptor; (3) Alteration in small intestine motility leading to small intestinal bacterial overgrowth and subsequent inhibition of IF-vitamin B12 complex absorption in the distal ileum; (4) Alteration in bile acid metabolism and reabsorption; (5) Increased liver accumulation of vitamin B12; and (6) Reduced IF secretion by gastric parietal cells
Use of medications that affect vitamin B12 absorption or metabolism including the bile acid resin cholestyramine (used to treat hypercholesterolemia), colchicine (used for acute gout) and many antibiotics such as neomycin and the anti-tuberculosis drug para-aminosalicylic acid[1]	Cholestyramine can chelate IF; colchicine and antibiotics can inhibit endocytosis of the IF-vitamin B12 complex
Bacterial overgrowth syndromes, ileal resection or gastrointestinal diseases such as terminal ileitis, celiac disease, inflammatory bowel disease, Crohn's disease and tropical sprue	Altered absorption of the IF-vitamin B12 complex in the terminal ileum; intestinal villous atrophy and mucosal injury (celiac disease, Crohn's disease and tropical sprue)
Intestinal parasitic infestations (often accompanied by eosinophilia) caused by the protozoan *Giardia lamblia* or the fish tapeworm *Diphyllobothrium latum*	Vitamin B12 malabsorption through vitamin B12 trapping by the parasites
Disorders of the exocrine pancreas or pancreatectomy	Insufficient pancreatic enzyme activity leads to a reduction in the proteolytic degradation of haptocorrin (mediated by pancreatic proteases in the small intestine); as a consequence, vitamin B12 remains bound to haptocorrin, cannot form the IF-vitamin B12 complex and is not available for absorption by the enterocytes in the distal ileum
Nitrous oxide anesthesia or recreational use of nitrous oxide	Irreversible oxidation and inactivation of the coenzyme form of vitamin B12 (methylcobalamin) at the active site of the vitamin B12-dependent methionine synthase reaction, resulting in increased levels of MMA and homocysteine
Inherited disorders affecting the sequential steps in the assimilation, transport and intracellular processing and metabolism of vitamin B12	Reduced expression, binding activity or affinity of receptors and proteins involved in transport, intracellular processing and metabolism of vitamin B12

[1]Unlike long-term use of proton-pump inhibitors, histamine H2-receptor antagonists or metformin, the frequency or duration of use of these drugs is usually insufficient to result in clinical vitamin B12 deficiency. H2RAs: Histamine H2-receptor antagonists; IF: Intrinsic factor; PPIs: Proton-pump inhibitors; MMA: Methylmalonic acid.

Johnson *et al*[19] found that the probability of being vitamin B12-deficient was significantly increased (2 times higher) in whites compared to African Americans. Another observational study by Carmel *et al*[23] reported that vitamin B12 deficiency was most common in elderly white men and least common in black and Asian American women. A large cross-sectional survey conducted in New Zealanders aged ≥ 15 years showed that Māori/Pacific and East/South-East Asian groups had the highest vitamin B12 levels, whereas those most at risk of low vitamin B12 status were South Asians, including people with ancestral origins in the Indian subcontinent[24]. Māori and Pacific Island groups were the least likely to have inadequate vitamin B12 intakes compared with New Zealand Europeans, while the latter group was more likely to have an adequate vitamin B12 status compared with South Asians[24]. Another study confirmed a higher prevalence of vitamin B12 deficiency in South Asians compared

with the general population[25].

METFORMIN-INDUCED VITAMIN B12 DEFICIENCY: CLINICAL EVIDENCE

More than 60 years after its first clinical use, metformin is still recommended as the first-line oral glucose-lowering drug in most clinical guidelines on the management of type 2 diabetes (T2D) thanks to its well-established long-term safety and efficacy profile[26]. Possible side effects of metformin include gastrointestinal intolerance[27] and the rare occurrence of lactic acidosis, which is most likely in the presence of moderate to severe chronic kidney disease. However, moderate to severe renal impairment is a major contraindication to the clinical use of metformin[28]. Metformin is usually well tolerated and effective in maintaining glucose control in the long-term [29]. Indeed, metformin is still the most widely used oral antihyperglycemic (insulin-sensitizing) agent, being prescribed to more than 100 million people worldwide, including patients with prediabetes, insulin resistance and polycystic ovary syndrome (PCOS)[30]. Yet, in recent decades, several observational studies, systematic reviews and meta-analyses have reported an association between long-term metformin therapy and biochemical vitamin B12 deficiency, including frank deficiency or borderline vitamin B12 status[31-37]. Evidence suggests that metformin impairs vitamin B12 status primarily in a dose- and duration-dependent manner (discussed later in the text).

The reported prevalence of vitamin B12 deficiency in metformin-treated patients with diabetes varies across studies, ranging from approximately 6% to 50%[31,38-41]. The first reports documenting this association were published in the late 1960s, when annual serum vitamin B12 testing was already suggested as a valid screening measure for early detection of vitamin B12 deficiency in patients on long-term metformin therapy[42,43]. However, the exact influence of both the dose and duration of metformin therapy on vitamin B12 status is still not entirely understood. A cross-sectional study conducted by de Groot-Kamphuis *et al*[38] found that patients with T2D using metformin had a significantly higher prevalence of vitamin B12 deficiency compared with patients not using metformin (14.1% *vs* 4.4%, with a median duration of metformin use of 4.9 years). Moreover, each 100 mg step in metformin dose increased by 8% the odds of having vitamin B12 deficiency, but metformin use did not predict the chance of having anemia or neuropathy[38]. A similar cross-sectional study conducted in 550 T2D patients using metformin (mean treatment duration of 64 mo and mean daily dose of 1306 mg) found that higher daily and cumulative doses of metformin (1 mg/d increase of daily dose and 10 g increase of cumulative dose) were strongly associated with lower HoloTC and cobalamin concentrations[39]. Nevertheless, authors did not find a relationship between the duration of metformin use and cobalamin/HoloTC concentrations[39]. A nested case-control study conducted in Hong Kong also suggested that an increased risk of vitamin B12 deficiency was associated with the current dose and duration of metformin therapy in patients with diabetes[44]. Authors found that each 1 g/d metformin dose increment conferred a more than 2-fold increased risk of developing vitamin B12 deficiency (adjusted odds ratio of 2.88, 95% confidence interval: 2.15-3.87; $P < 0.001$). In patients using metformin for 3 years or more, the adjusted odds ratio was 2.39 (95% confidence interval: 1.46-3.91; $P = 0.001$) compared with those receiving metformin for less than 3 years[44].

Shivaprasad *et al*[45] recently conducted a prospective observational study to assess the combined effect of both dose and duration of metformin therapy on vitamin B12 levels in 2887 patients with T2D. They found vitamin B12 levels of < 200 pg/mL and between 200 and 300 pg/mL in 24.5% and 34.5% of metformin users, respectively. The percentages were significantly higher than those observed in non-metformin users (17.3% and 22.6%, respectively). To quantify metformin usage, authors defined a "metformin usage index" (MUI) as the product of the daily metformin dose (mg) and its duration (years) divided by 1000. Participants who were not on continuous metformin therapy for at least 6 mo prior to recruitment were included in a non-metformin user group. Interestingly, there was a significant association between a MUI value of > 5 and a high risk of vitamin B12 deficiency. Multistep logistic regression analysis adjusted for confounding variables (age, duration of T2D, body mass index, and glycated hemoglobin) found the highest risk of developing vitamin B12 deficiency in patients with a MUI value of > 15, followed by patients with a MUI value of > 10. The lowest risk was found in T2D patients with a MUI value of < 5[45]. Therefore, MUI may be a valid tool to identify individuals at increased risk of vitamin B12 deficiency among T2D patients on continuous metformin therapy for at least 6 mo.

Even though definitive screening guidelines are lacking, the 2021 American Diabetes Association Standards of Medical Care in Diabetes recommend to consider a periodic assessment of vitamin B12 levels in patients with long-term metformin use, including those with prediabetes, peripheral neuropathy or anemia. This recommendation is based on a grade B level of evidence deriving from well-conducted case-control studies, prospective cohort studies and meta-analyses of cohort studies[46], including a report from the Diabetes Prevention Program Outcomes Study (DPPOS) published in *The Journal of Clinical Endocrinology & Metabolism* in 2016 by Aroda *et al* [32]. The design of the latter study consisted of a secondary analysis from the Diabetes Prevention Program (DPP)/DPPOS involving over 2000 patients across 27 centers in the United States. Participants with elevated fasting blood glucose, impaired glucose tolerance, and overweight or obesity were assigned to the placebo group (n = 1082) or to the metformin group (850 mg twice daily; n = 1073) for a mean follow-up of 3.2 years. Participants in the metformin group received open-label metformin for an additional 9 years. Authors found that low serum vitamin B12 levels (≤ 203 pg/mL) occurred with a significantly higher frequency in the metformin group than in the placebo group at 5 years (4.3% *vs* 2.3%). Furthermore, combined low and borderline-low vitamin B12 (≤ 298 pg/mL) was significantly more frequent in metformin group at 5 and 13 years. Importantly, authors reported that approximately 50% of participants with low vitamin B12 levels in their cohort had concurrently increased homocysteine levels, suggesting the presence of a true tissue deficiency of vitamin B12. Moreover, years of metformin use were associated with an increased risk of vitamin B12 deficiency. When the metformin and placebo groups were combined, the odds ratio associated with vitamin B12 deficiency per year of metformin use was 1.13 (95% confidence interval: 1.06-1.20) after adjusting for confounders such as age, sex, baseline body mass index, weight change, diabetes status, and prescription of acid suppression therapy[32]. The prevalence of anemia was higher in the metformin group, but did not differ by vitamin B12 status. The prevalence of neuropathy was significantly higher among metformin group participants with low vitamin B12 levels compared with metformin-treated participants with normal or borderline vitamin B12 levels[32]. In line with those findings, a cross-sectional study by Kim *et al*[33] found that metformin use in T2D patients for at least 6 mo and at a dose of ≥ 1500 mg/d may represent a major factor related to vitamin B12 deficiency. Of note, authors found that a metformin dose of ≥ 2000 mg was associated with the highest risk of vitamin B12 deficiency. Compared with the group taking a daily metformin dose of < 1000 mg, the adjusted odds ratios for 1000-1500 mg, 1500-2000 mg, and ≥ 2000 mg groups were 1.72 (P = 0.080), 3.34 (P < 0.001) and 8.67 (P < 0.001), respectively. Moreover, serum homocysteine levels were negatively correlated with vitamin B12 levels, suggesting that vitamin B12 deficiency induced by metformin may occur at the tissue level[33].

A recent retrospective study conducted in a large cohort of adult patients (n = 13489) who had received metformin for more than 1 year aimed to assess the appropriateness and benefits of screening recommendations for vitamin B12 deficiency[47]. The mean time between metformin initiation and incidence of vitamin B12 deficiency was 5.3 years. An older patient subgroup (> 65 years of age) had a significantly higher vitamin B12 deficiency rate compared with younger patients (4.2% *vs* 2.5%). In multivariable logistic regression models, older age was the only factor associated with vitamin B12 deficiency, while African-American ethnicity almost reached statistical significance as a protective factor. These results suggest that patients who have been using metformin for more than 5 years and patients older than 65 are at increased risk for vitamin B12 deficiency. Therefore, authors concluded that screening for vitamin B12 deficiency might be considered in such populations even if they are asymptomatic for the deficiency[47].

A prospective case-control study conducted in T2D patients with concurrent symptomatic peripheral neuropathy found that patients who had received metformin for more than 6 mo with a mean cumulative metformin exposure of 3389.5 g, compared with those without metformin exposure, had lower cobalamin levels and higher fasting MMA and homocysteine levels accompanied by more severe peripheral neuropathy (assessed by clinical and electrophysiological markers)[48]. The cumulative metformin dose was inversely correlated with serum vitamin B12 and positively correlated with fasting serum MMA and homocysteine. In addition, the median Toronto Clinical Scoring System (TCSS) and Neuropathy Impairment Score (NIS) total scores were both significantly higher in the metformin-treated group and had a strong positive correlation with increasing cumulative metformin dose[48]. In keeping with those findings, a post-hoc analysis of a randomized controlled 4.3-year trial conducted in insulin-treated T2D patients reported that addition of metformin not only reduced serum vitamin B12 levels, but also gradually increased serum MMA

levels[49]. In metformin users, the increase in MMA levels was also associated with significant worsening of symptoms of neuropathy, assessed by a validated neuropathy score[49].

A possible explanation for the delayed occurrence of biochemical or clinical vitamin B12 deficiency in patients on metformin therapy relies on the low daily requirement of vitamin B12 (approximately 2.4 μg/d) and on its substantial hepatic storage of around 2500 μg. Indeed, clinical manifestations of vitamin B12 deficiency can become evident upon depletion of the body stores to as little as 5%-10%, which may occur several years (up to 10 years) after the initial exposure to metformin or to other risk factors or conditions predisposing to vitamin B12 deficiency[50]. However, older adults may exhibit depleted vitamin B12 stores[51] and may therefore be particularly susceptible to vitamin B12 deficiency even after a short-term period of metformin therapy. For instance, Leung *et al*[52] showed that short-term (3-mo) metformin use decreased plasma levels of total cobalamin, total haptocorrin and haptocorrin-bound cobalamin in an elderly diabetic population.

Evidence also suggests that acid-suppressing medications such as histamine H2-receptor antagonists (H2RAs) and proton-pump inhibitors (PPIs) can interfere with vitamin B12 absorption by reducing the release of dietary vitamin B12 from food proteins[53]. Notably, Long *et al*[54] reported that the concomitant use of metformin and PPIs in patients with T2D may increase the risk of vitamin B12 deficiency by exerting further deleterious effects on vitamin B12 status. This has relevant clinical implications, given that approximately 40% of T2D patients are reported to experience symptomatic gastroesophageal reflux disease (GERD), and PPIs and H2RAs represent the most widely prescribed drugs for treatment of GERD in this population[53].

Metformin-induced vitamin B12 deficiency: implications for diabetic neuropathy

Metformin-induced vitamin B12 deficiency (also known as MICD or metformin-induced cobalamin deficiency) can exacerbate nerve damage in diabetic patients with preexisting neuropathy, resulting in the development of a mixed "diabetic and MICD-related neuropathy". Hashem *et al*[55] recently conducted a case-control, prospective, observational study in 150 adults with T2D and diabetic peripheral neuropathy (DPN) to establish whether metformin represents a risk factor for DPN. The study cohort included 75 patients who received metformin for the previous 6 mo or more and 75 patients who did not receive metformin for the previous 6 mo but had received other oral antihyperglycemic drugs. Compared with the control patients, the metformin-treated patients had significantly higher homocysteine and MMA levels, along with significantly lower plasma cobalamin levels (222 pmol/L *vs* 471 pmol/L; $P < 0.001$). Moreover, metformin-treated patients had a significantly higher frequency of moderate to severe DPN and higher TCSS total scores. Spearman's correlation revealed a significant negative correlation between plasma cobalamin levels and higher metformin doses, as well as a significant positive correlation between TCSS and increased metformin dose. Metformin-treated patients also showed significantly lower median conduction velocity and sensory nerve action potentials for superficial peroneal and sural nerves. In addition, the severity of DPN was inversely related to plasma cobalamin levels and directly related to higher levels of both homocysteine and MMA. Importantly, multivariate logistic regression analysis of independent predictors of DPN in metformin-treated patients revealed that longer duration of diabetes and metformin therapy were significantly associated with a greater incidence of DPN. Larger doses and longer duration of metformin therapy were also independent predictors of DPN[55].

In a recent 12-mo, randomized, double-blind, placebo-controlled trial, 90 adults with T2D on metformin therapy for at least 4 years and with both diabetic peripheral and autonomic neuropathy were randomized to receive vitamin B12 (1 mg of oral methylcobalamin) or placebo on a daily basis[56]. All participants had baseline vitamin B12 levels of < 400 pmol/L. Compared with placebo, vitamin B12 supplementation led to a significant increase in vitamin B12 levels (from 232.0 ± 71.8 pmol/L at baseline to 776.7 ± 242.3 pmol/L at follow-up) that was accompanied by a significant improvement in Michigan Neuropathy Screening Instrument Questionnaire, quality of life and pain scores, vibration perception threshold, electrochemical skin conductance in the feet, and sural nerve conduction velocity and amplitude[56]. Overall, these findings highlight the importance of diagnosing and correcting vitamin B12 deficiency in metformin-treated patients with diabetes and neuropathy in order to prevent or halt the exacerbation and progression of nerve damage.

POTENTIAL PATHOPHYSIOLOGICAL MECHANISMS OF METFORMIN-INDUCED VITAMIN B12 DEFICIENCY

The exact mechanisms underlying metformin-induced vitamin B12 deficiency are still not fully understood[37]. However, the mechanisms are thought to cause vitamin B12 deficiency mainly through altered vitamin B12 absorption and metabolism (Figure 1) [1,57-60]. Proposed mechanisms accounting for metformin-induced vitamin B12 deficiency include: (1) Interference with calcium-dependent binding of the IF-vitamin B12 complex to the cubilin receptor on enterocytes at the ileum level and/or interaction with the cubilin endocytic receptor; (2) Alteration in small intestine motility, leading to small intestinal bacterial overgrowth and subsequent inhibition of IF-vitamin B12 complex absorption in the distal ileum; (3) Alteration in bile acid metabolism and reabsorption, resulting in impaired enterohepatic circulation of vitamin B12; (4) Increased liver accumulation of vitamin B12, resulting in altered tissue distribution and metabolism of vitamin B12; and (5) Reduced IF secretion by gastric parietal cells. Of note, inhibition of calcium-dependent absorption of the IF-vitamin B12 complex at the terminal ileum has been increasingly recognized as the most plausible mechanism accounting for metformin-induced vitamin B12 deficiency. Indeed, the inhibitory effect is reversed by calcium supplementation[61].

VITAMIN B12 DEFICIENCY IN METFORMIN-TREATED PATIENTS: PROPOSED CRITERIA FOR A COST-EFFECTIVE SCREENING

To date, no definite guidelines are available for the screening of vitamin B12 deficiency in patients taking metformin. Thus, vitamin B12 deficiency remains frequently unrecognized in such patients. According to the British Society for Haematology guidelines for diagnosis and treatment of vitamin B12 deficiency, no definitive advice can be given on the desirable frequency of measurement of serum vitamin B12 in patients with T2D on metformin therapy. However, the same guidelines recommend to check serum vitamin B12 levels in the presence of strong clinical suspicion of deficiency[62].

Based on the current evidence, we therefore propose a list of criteria for a cost-effective vitamin B12 deficiency screening in metformin-treated patients. The criteria could serve as a practical guide for identifying individuals at high risk for vitamin B12 deficiency who may require vitamin B12 supplementation as well as a periodic assessment of their cobalamin status (Table 2). Of note, we suggest to consider screening for vitamin B12 deficiency in selected individuals even if they are asymptomatic for deficiency. The proposed screening criteria may be useful to prevent the development or worsening of the clinical consequences of vitamin B12 deficiency, particularly anemia and peripheral neuropathy, by allowing for a prompt diagnosis and treatment of vitamin B12 deficiency.

Apart from metformin-treated patients with insulin resistance, prediabetes, T2D and PCOS, another subgroup in which routine screening for vitamin B12 deficiency may be considered includes patients with type 1 diabetes (T1D) taking metformin as non-insulin adjunct therapy, especially in the presence of peripheral neuropathy or unexplained anemia. In this regard, it is also worth reminding that T1D patients are at increased risk for other autoimmune diseases such as autoimmune gastritis and pernicious anemia, which can independently lead to the development of vitamin B12 deficiency[63,64].

TREATMENT OF VITAMIN B12 DEFICIENCY

In selected individuals who are at higher risk for marginal vitamin B12 insufficiency, the goal is first of all to prevent the development of frank vitamin B12 deficiency, then to treat such deficiency by adequate repletion when it occurs[5]. This approach allows for prevention of clinical consequences of vitamin B12 deficiency, including megaloblastic anemia and neurologic manifestations such as peripheral neuropathy.

The recommended dietary allowance of vitamin B12 for subjects without malabsorption has been set at approximately 2.4 μg/d for adult men and non-pregnant women (2.6 μg/d for pregnant women)[8], even though a daily intake of 4-7 μg has been associated with lower serum MMA levels[65]. There is no defined tolerable upper intake level of vitamin B12[66]. As vitamin B12 is relatively

Table 2 Proposed criteria for cost-effective screening and subsequent intermittent periodic testing of vitamin B12 status in metformin-treated patients

Proposed criteria
(1) A comprehensive assessment of vitamin B12 status aimed to accurately detect a true tissue vitamin B12 deficiency should include at least one biomarker of circulating vitamin B12 (total vitamin B12 or HoloTC) coupled with one functional (metabolic) biomarker of vitamin B12 status (MMA and/or total homocysteine). A recent complete blood count is also recommended
(2) Screening for vitamin B12 deficiency should be performed in the presence of one or more of the following risk factors or conditions: (a) Strong clinical suspicion of deficiency: clinical evidence of vitamin B12 deficiency, including unexplained macrocytic anemia, neurological symptoms and peripheral neuropathy[1]; (b) Preexisting diabetic peripheral and/or autonomic neuropathy[2]; (c) Duration of metformin treatment ≥ 5 yr; (d) Older adults: age ≥ 65 yr; (e) High cumulative metformin exposure defined by a MUI value of > 5 (this criterion applies to patients with type 2 diabetes treated with metformin for at least 6 mo)[3]; (f) Metformin dose of ≥ 1500 mg/d for a duration of at least 6 mo (the highest risk of vitamin B12 deficiency has been observed with a daily metformin dose of ≥ 2000 mg); (g) Concomitant long-term use (≥ 12 mo) of acid-suppressing medications such as PPIs and H2RAs; and (h) Concomitant presence of risk factors or comorbidities associated with an increased risk of vitamin B12 deficiency (reviewed in Table 1) warrants screening for deficiency based on clinical judgement

[1]Based on results from Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study[32], peripheral neuropathy refers to monofilament-defined neuropathy (detection of an abnormal monofilament examination).

[2]Screening for vitamin B12 deficiency should be routinely performed in metformin-treated diabetic patients with a preexisting diabetic peripheral and/or autonomic neuropathy. Once diagnosed, metformin-induced vitamin B12 deficiency should be corrected promptly in such patients in order to counteract the exacerbation of nerve damage and prevent the development or progression of a mixed "diabetic and metformin-induced cobalamin deficiency-related neuropathy".

[3]Metformin Usage Index (MUI) is defined as the product of the daily metformin dose (mg) and its duration (yr) divided by 1000. For example, 1000 mg of metformin used for a duration of 1 yr is equivalent to 1 MUI (1000 × 1/1000 = 1 MUI). This criterion applies to patients with type 2 diabetes treated with metformin for at least 6 mo, based on the results from the prospective observational study conducted by Shivaprasad *et al*[45]. H2RAs: Histamine H2-receptor antagonists; HoloTC: Holotranscobalamin; MMA: Methylmalonic acid; MUI: Metformin Usage Index; PPIs: Proton-pump inhibitors.

inexpensive, easy to administer, safe and well tolerated, a personalized approach to meet the need of the individual patient is generally deemed as harmless[5].

Synthetic vitamin B12 has long been available in the form of cyanocobalamin, both for oral and injectable use. Subsequently, naturally occurring forms of vitamin B12 have become commercially available, including hydroxycobalamin, methylcobalamin and adenosylcobalamin[67]. Hydroxycobalamin is commonly used in Europe at intervals of approximately 2-3 mo, as it appears to have better retention than cyanocobalamin[5]. Oral and intramuscular routes of vitamin B12 administration are frequently used for treatment of vitamin B12 deficiency. The efficacy of alternative routes of vitamin B12 administration (*e.g.*, intranasal or sublingual administration) to treat vitamin B12 deficiency has also been reported[68,69]. Indeed, a recent 12-wk randomized intervention trial showed that sublingual administration of 50 μg/d cyanocobalamin restored adequate serum concentrations of vitamin B12 in vegans and vegetarians with a marginal cobalamin deficiency[69]. After intramuscular injection of cyanocobalamin, about 10%-15% of the total administered dose (*e.g.*, 150 μg of 1000 μg) is ultimately retained in the body, primarily through storage in the liver, although a remarkable interindividual variability in vitamin B12 retention capacity has been reported[5,8]. Therefore, intramuscular injection of vitamin B12 in high doses allows for rapid replenishment of body stores of the vitamin. High-dose oral vitamin B12 supplementation is an effective alternative to parenteral treatment (*e.g.*, in patients who do not tolerate intramuscular injections). Approximately 0.5%-4% of an oral vitamin B12 dose is usually absorbed[5]; for example, an oral dose of 1000 μg will deliver on average 5-40 μg of vitamin B12[5], which adequately meet the recommended daily intake of vitamin B12.

However, route of administration and duration of treatment primarily depend on the underlying etiology and severity of vitamin B12 deficiency. In subjects with vitamin B12 deficiency caused by malabsorption rather than by inadequate dietary intake, high-dose vitamin B12 administration should be initiated as follows: (1) Intramuscular injection of 1000 μg of cyanocobalamin or hydroxycobalamin daily or every other day for 1 wk, followed by weekly injections up to 8 wk, and every 3-4 wk afterward; or (2) Oral administration of cyanocobalamin in high daily doses (2000 μg/d) until remission, and 1000-2000 μg daily afterward[5]. Conversely, patients who are vitamin B12 deficient because of low dietary intake will require loading with high-dose vitamin B12 to restore tissue levels over 3-4 mo; subsequently, smaller doses of at least 6 μg/d will generally suffice, as conservation of biliary vitamin B12 is possible *via* the enterohepatic recycling and the physiologically highly efficient reabsorption of biliary vitamin B12[5]. Patients lacking IF (*e.g.*, those with true pernicious anemia) cannot reabsorb the vitamin B12 lost in bile (which varies from 3 μg/d to 9 μg/d), and 100-300 μg of vitamin B12 should therefore be retained monthly to maintain tissue

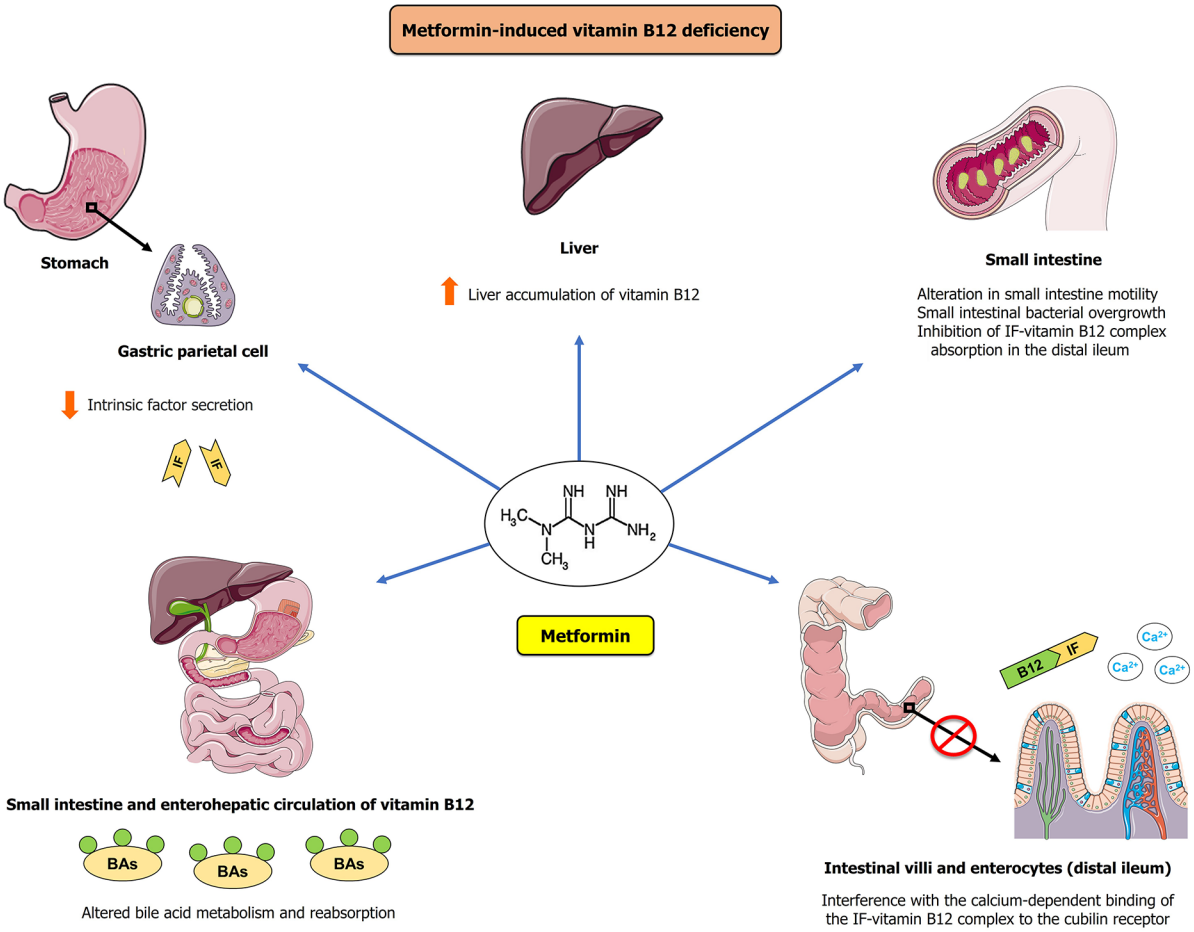

Figure 1 Postulated mechanisms accounting for metformin-induced vitamin B12 deficiency. Metformin may cause vitamin B12 deficiency through one or more of the following mechanisms: (1) Interference with the calcium-dependent binding of the intrinsic factor (IF)-vitamin B12 complex to the cubilin receptor on enterocytes at the ileum level and/or interaction with the cubilin endocytic receptor; (2) Alteration in bile acid metabolism and reabsorption, resulting in impaired enterohepatic circulation of vitamin B12; (3) Reduced IF secretion by gastric parietal cells; (4) Increased liver accumulation of vitamin B12, resulting in altered tissue distribution and metabolism of vitamin B12; and (5) Alteration in small intestine motility, resulting in small intestinal bacterial overgrowth and subsequent inhibition of IF-vitamin B12 complex absorption in the distal ileum. B12: Vitamin B12; BAs: Bile acids; IF: Intrinsic factor.

stores[5]. In this regard, it is worth mentioning that about 1% of oral vitamin B12 can be absorbed in the small intestine through a passive diffusion pathway that is independent of IF and remains unaffected in patients with pernicious anemia[70]. Thus, high oral doses of 1000-2000 μg/d vitamin B12 can meet the estimated daily requirement of 2.4 μg/d even in patients with impaired IF secretion. Notwithstanding, there are arguments against the use of oral vitamin B12 in severely deficient individuals with poor intestinal absorption (particularly in those with pernicious anemia), in whom intramuscular injection may be preferred to assure effective treatment[62]. Further-more, patients with vitamin B12 deficiency presenting with severe neurologic manifestations should be treated by intramuscular injection of 1000 μg of vitamin B12 on alternate days until no further improvement is noted[62]. With regard to the duration of vitamin B12 supplementation, patients with an irreversible cause of vitamin B12 deficiency should be treated indefinitely, and those with a reversible cause should be treated until the deficiency is corrected and the symptoms resolve[8,71]. In the presence of concomitant folate deficiency, vitamin B12 deficiency should be corrected first to prevent subacute combined degeneration of the spinal cord [8,71].

Treatment of metformin-induced vitamin B12 deficiency

According to the British Society for Haematology guidelines for diagnosis and treatment of vitamin B12 deficiency, no recommendations can be currently given on prophylactic administration with oral vitamin B12 in patients using metformin[62]. Despite the lack of definitive guidelines or recommendations on treatment of metformin-induced vitamin B12 deficiency, patients using metformin with concomitant vitamin B12 deficiency should receive cobalamin supplementation aimed to

correct this deficiency and prevent the related risk of peripheral nerve damage and/or other clinical consequences. Importantly, prompt vitamin B12 administration should be considered, particularly in metformin-treated patients with vitamin B12 deficiency accompanied by neurologic and/or hematologic manifestations such as peripheral neuropathy and megaloblastic anemia.

Although treatment of vitamin B12 deficiency in metformin-treated patients may certainly be cost-effective, an issue that still needs to be addressed relies on the fact that the most appropriate repletion method has not yet been clearly defined. As metformin appears to interfere with vitamin B12 absorption in the small intestine through different mechanisms, intramuscular or sublingual routes of administration may theoretically be superior to oral supplementation for treatment of metformin-induced vitamin B12 through bypassing the gastrointestinal tract and intestinal absorption. However, it is also plausible that high-dose oral vitamin B12 supplementation may be as effective as other routes of administration in overcoming the malabsorption caused by metformin and adequately correcting the cobalamin deficiency. Future studies are therefore warranted to establish the most effective and convenient route of administration of vitamin B12 in metformin-treated patients with concomitant vitamin B12 deficiency. As oral calcium supplementation has been shown to reverse vitamin B12 malabsorption caused by metformin[61], it would also be interesting to investigate whether this therapeutic approach may represent an alternative, safe and effective tool to treat metformin-induced vitamin B12 deficiency.

CONCLUSION

Several recent observational studies and meta-analyses have reported a significant association between long-term metformin therapy and an increased prevalence of vitamin B12 deficiency. The exact mechanisms accounting for vitamin B12 deficiency caused by metformin are still not entirely clear, although it is highly plausible that such mechanisms are related to the impaired vitamin B12 absorption in the small intestine.

Given the high global prevalence of diabetes, which affects more than 460 million people worldwide[72], the widespread use of metformin as an insulin-sensitizing agent for treatment of insulin resistance, prediabetes, T2D and PCOS, as well as the chronic nature of treatment of such conditions, it is important to recognize vitamin B12 deficiency as a potential adverse consequence of long-term and high-dose metformin therapy. As no definite guidelines are currently available for vitamin B12 deficiency screening in metformin-treated patients, this deficiency remains often unrecognized in such individuals.

Therefore, we believe that initial screening and subsequent intermittent periodic testing of vitamin B12 status in selected patients treated with metformin may be cost-effective and should be considered in order to promptly identify and correct vitamin B12 deficiency. In this regard, we have proposed a list of criteria for a cost-effective screening and subsequent intermittent periodic testing of vitamin B12 status in metformin-treated patients who are at high risk for deficiency (Table 2). The criteria include: (1) Strong clinical suspicion of vitamin B12 deficiency; (2) Preexisting diabetic peripheral and/or autonomic neuropathy; (3) Duration of metformin therapy of ≥ 5 years; (4) Age ≥ 65 years; (5) High cumulative metformin exposure defined by a MUI value of > 5; (6) A metformin dose of ≥ 1500 mg/d for a duration of at least 6 mo; (7) Concomitant long-term use (≥ 12 mo) of acid-suppressing medications; and (8) Concomitant presence of risk factors or comorbidities associated with an increased risk of vitamin B12 deficiency (reviewed in Table 1). Yet, we acknowledge that these criteria need to be validated in large prospective studies. Further studies are also needed to ascertain the desirable frequency of assessment of vitamin B12 status in patients receiving metformin therapy. Thus, additional research is required to develop protocols for screening, prevention and treatment of metformin-induced vitamin B12.

In patients with metformin-induced vitamin B12 deficiency, vitamin B12 supplementation offers a simple, safe and effective means of preventing the development or the worsening of peripheral nerve damage, anemia and/or other clinical manifestations of vitamin B12 deficiency. To date, there are no specific guidelines for the treatment of vitamin B12 deficiency induced by metformin therapy. Hence, clinicians should correct vitamin B12 deficiency in patients treated with metformin following the British Society for Haematology guidelines for diagnosis and treatment of vitamin B12 deficiency[62]. Likewise, these remarks apply to metformin-treated diabetic patients with preexisting diabetic peripheral and/or autonomic neuropathy who may

experience an exacerbation of nerve damage as well as a substantial deterioration of neuropathy resulting from the concomitant development of MICD. In light of the highly favorable safety and efficacy profile of metformin as an insulin-sensitizing agent, we certainly recommend against discontinuing this drug in patients with newly diagnosed vitamin B12 deficiency. However, it is prudent to periodically assess vitamin B12 status in metformin-treated patients who could remain at increased risk of vitamin B12 deficiency even after an adequate vitamin B12 supplementation.

ACKNOWLEDGEMENTS

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REFERENCES

1 **Obeid R**, Heil SG, Verhoeven MMA, van den Heuvel EGHM, de Groot LCPGM, Eussen SJPM. Vitamin B12 Intake From Animal Foods, Biomarkers, and Health Aspects. *Front Nutr* 2019; **6**: 93 [PMID: 31316992 DOI: 10.3389/fnut.2019.00093]
2 **Beedholm-Ebsen R**, van de Wetering K, Hardlei T, Nexø E, Borst P, Moestrup SK. Identification of multidrug resistance protein 1 (MRP1/ABCC1) as a molecular gate for cellular export of cobalamin. *Blood* 2010; **115**: 1632-1639 [PMID: 19897579 DOI: 10.1182/blood-2009-07-232587]
3 **Seetharam B**, Li N. Transcobalamin II and its cell surface receptor. *Vitam Horm* 2000; **59**: 337-366 [PMID: 10714245 DOI: 10.1016/s0083-6729(00)59012-8]
4 **Ankar A**, Kumar A. Vitamin B12 Deficiency. 2020 Jun 7. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [PMID: 28722952]
5 **Green R**, Allen LH, Bjørke-Monsen AL, Brito A, Guéant JL, Miller JW, Molloy AM, Nexo E, Stabler S, Toh BH, Ueland PM, Yajnik C. Vitamin B_{12} deficiency. *Nat Rev Dis Primers* 2017; **3**: 17040 [PMID: 28660890 DOI: 10.1038/nrdp.2017.40]
6 **Konda M**, Godbole A, Pandey S, Sasapu A. Vitamin B12 deficiency mimicking acute leukemia. *Proc (Bayl Univ Med Cent)* 2019; **32**: 589-592 [PMID: 31656431 DOI: 10.1080/08998280.2019.1641045]
7 **Kumar N**. Neurologic aspects of cobalamin (B12) deficiency. *Handb Clin Neurol* 2014; **120**: 915-926 [PMID: 24365360 DOI: 10.1016/B978-0-7020-4087-0.00060-7]
8 **Langan RC**, Goodbred AJ. Vitamin B12 Deficiency: Recognition and Management. *Am Fam Physician* 2017; **96**: 384-389 [PMID: 28925645]
9 **Kibirige D**, Mwebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified? *J Diabetes Metab Disord* 2013; **12**: 17 [PMID: 23651730 DOI: 10.1186/2251-6581-12-17]
10 **Bell DS**. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. *South Med J* 2010; **103**: 265-267 [PMID: 20134380 DOI: 10.1097/SMJ.0b013e3181ce0e4d]
11 **Köbe T**, Witte AV, Schnelle A, Grittner U, Tesky VA, Pantel J, Schuchardt JP, Hahn A, Bohlken J, Rujescu D, Flöel A. Vitamin B-12 concentration, memory performance, and hippocampal structure in patients with mild cognitive impairment. *Am J Clin Nutr* 2016; **103**: 1045-1054 [PMID: 26912492 DOI: 10.3945/ajcn.115.116970]
12 **Hannibal L**, Lysne V, Bjørke-Monsen AL, Behringer S, Grünert SC, Spiekerkoetter U, Jacobsen DW, Blom HJ. Biomarkers and Algorithms for the Diagnosis of Vitamin B12 Deficiency. *Front Mol Biosci* 2016; **3**: 27 [PMID: 27446930 DOI: 10.3389/fmolb.2016.00027]
13 **Yetley EA**, Pfeiffer CM, Phinney KW, Bailey RL, Blackmore S, Bock JL, Brody LC, Carmel R, Curtin LR, Durazo-Arvizu RA, Eckfeldt JH, Green R, Gregory JF 3rd, Hoofnagle AN, Jacobsen DW, Jacques PF, Lacher DA, Molloy AM, Massaro J, Mills JL, Nexo E, Rader JI, Selhub J, Sempos C, Shane B, Stabler S, Stover P, Tamura T, Tedstone A, Thorpe SJ, Coates PM, Johnson CL, Picciano MF. Biomarkers of vitamin B-12 status in NHANES: a roundtable summary. *Am J Clin Nutr* 2011; **94**: 313S-321S [PMID: 21593512 DOI: 10.3945/ajcn.111.013243]
14 **Solomon LR**. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. *Blood* 2005; **105**: 978-85; author reply 1137 [PMID: 15466926 DOI: 10.1182/blood-2004-04-1641]
15 **Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate**, Other B Vitamins, and Choline. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B 6, Folate, Vitamin B 12, Pantothenic Acid, Biotin, and Choline. 1998 [PMID: 23193625 DOI: 10.17226/6015]
16 **Lupoli R**, Lembo E, Saldalamacchia G, Avola CK, Angrisani L, Capaldo B. Bariatric surgery and long-term nutritional issues. *World J Diabetes* 2017; **8**: 464-474 [PMID: 29204255 DOI: 10.4239/wjd.v8.i11.464]
17 **Pennypacker LC**, Allen RH, Kelly JP, Matthews LM, Grigsby J, Kaye K, Lindenbaum J, Stabler SP. High prevalence of cobalamin deficiency in elderly outpatients. *J Am Geriatr Soc* 1992; **40**: 1197-1204 [PMID: 1447433]

18 **Baik HW**, Russell RM. Vitamin B12 deficiency in the elderly. *Annu Rev Nutr* 1999; **19**: 357-377 [PMID: 10448529 DOI: 10.1146/annurev.nutr.19.1.357]

19 **Johnson MA**, Hausman DB, Davey A, Poon LW, Allen RH, Stabler SP; Georgia Centenarian Study. Vitamin B12 deficiency in African American and white octogenarians and centenarians in Georgia. *J Nutr Health Aging* 2010; **14**: 339-345 [PMID: 20424799 DOI: 10.1007/s12603-010-0077-y]

20 **Murphy MM**, Molloy AM, Ueland PM, Fernandez-Ballart JD, Schneede J, Arija V, Scott JM. Longitudinal study of the effect of pregnancy on maternal and fetal cobalamin status in healthy women and their offspring. *J Nutr* 2007; **137**: 1863-1867 [PMID: 17634256 DOI: 10.1093/jn/137.8.1863]

21 **Milman N**, Byg KE, Bergholt T, Eriksen L, Hvas AM. Cobalamin status during normal pregnancy and postpartum: a longitudinal study comprising 406 Danish women. *Eur J Haematol* 2006; **76**: 521-525 [PMID: 16548919 DOI: 10.1111/j.0902-4441.2006.t01-1-EJH2550.x]

22 **Greibe E**, Andreasen BH, Lildballe DL, Morkbak AL, Hvas AM, Nexo E. Uptake of cobalamin and markers of cobalamin status: a longitudinal study of healthy pregnant women. *Clin Chem Lab Med* 2011; **49**: 1877-1882 [PMID: 21875397 DOI: 10.1515/CCLM.2011.682]

23 **Carmel R**, Green R, Jacobsen DW, Rasmussen K, Florea M, Azen C. Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. *Am J Clin Nutr* 1999; **70**: 904-910 [PMID: 10539753 DOI: 10.1093/ajcn/70.5.904]

24 **Devi A**, Rush E, Harper M, Venn B. Vitamin B12 Status of Various Ethnic Groups Living in New Zealand: An Analysis of the Adult Nutrition Survey 2008/2009. *Nutrients* 2018; **10** [PMID: 29414857 DOI: 10.3390/nu10020181]

25 **Gupta AK**, Damji A, Uppaluri A. Vitamin B12 deficiency. Prevalence among South Asians at a Toronto clinic. *Can Fam Physician* 2004; **50**: 743-747 [PMID: 15171677]

26 **Marshall SM**. 60 years of metformin use: a glance at the past and a look to the future. *Diabetologia* 2017; **60**: 1561-1565 [PMID: 28776085 DOI: 10.1007/s00125-017-4343-y]

27 **Bonnet F**, Scheen A. Understanding and overcoming metformin gastrointestinal intolerance. *Diabetes Obes Metab* 2017; **19**: 473-481 [PMID: 27987248 DOI: 10.1111/dom.12854]

28 **DeFronzo R**, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism* 2016; **65**: 20-29 [PMID: 26773926 DOI: 10.1016/j.metabol.2015.10.014]

29 **Ito H**, Ishida H, Takeuchi Y, Antoku S, Abe M, Mifune M, Togane M. Long-term effect of metformin on blood glucose control in non-obese patients with type 2 diabetes mellitus. *Nutr Metab (Lond)* 2010; **7**: 83 [PMID: 21070671 DOI: 10.1186/1743-7075-7-83]

30 **Viollet B**, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)* 2012; **122**: 253-270 [PMID: 22117616 DOI: 10.1042/CS20110386]

31 **Chapman LE**, Darling AL, Brown JE. Association between metformin and vitamin B_{12} deficiency in patients with type 2 diabetes: A systematic review and meta-analysis. *Diabetes Metab* 2016; **42**: 316-327 [PMID: 27130885 DOI: 10.1016/j.diabet.2016.03.008]

32 **Aroda VR**, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, Bray GA, Schade DS, Temprosa MG, White NH, Crandall JP; Diabetes Prevention Program Research Group. Long-term Metformin Use and Vitamin B12 Deficiency in the Diabetes Prevention Program Outcomes Study. *J Clin Endocrinol Metab* 2016; **101**: 1754-1761 [PMID: 26900641 DOI: 10.1210/jc.2015-3754]

33 **Kim J**, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes. *Medicine (Baltimore)* 2019; **98**: e17918 [PMID: 31725641 DOI: 10.1097/MD.0000000000017918]

34 **Kancherla V**, Elliott JL Jr, Patel BB, Holland NW, Johnson TM 2nd, Khakharia A, Phillips LS, Oakley GP Jr, Vaughan CP. Long-term Metformin Therapy and Monitoring for Vitamin B12 Deficiency Among Older Veterans. *J Am Geriatr Soc* 2017; **65**: 1061-1066 [PMID: 28182265 DOI: 10.1111/jgs.14761]

35 **Niafar M**, Hai F, Porhomayon J, Nader ND. The role of metformin on vitamin B12 deficiency: a meta-analysis review. *Intern Emerg Med* 2015; **10**: 93-102 [PMID: 25502588 DOI: 10.1007/s11739-014-1157-5]

36 **Liu Q**, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: systematic review. *PLoS One* 2014; **9**: e100379 [PMID: 24959880 DOI: 10.1371/journal.pone.0100379]

37 **Mazokopakis EE**, Starakis IK. Recommendations for diagnosis and management of metformin-induced vitamin B12 (Cbl) deficiency. *Diabetes Res Clin Pract* 2012; **97**: 359-367 [PMID: 22770998 DOI: 10.1016/j.diabres.2012.06.001]

38 **de Groot-Kamphuis DM**, van Dijk PR, Groenier KH, Houweling ST, Bilo HJ, Kleefstra N. Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin. *Neth J Med* 2013; **71**: 386-390 [PMID: 24038568]

39 **Beulens JW**, Hart HE, Kuijs R, Kooijman-Buiting AM, Rutten GE. Influence of duration and dose of metformin on cobalamin deficiency in type 2 diabetes patients using metformin. *Acta Diabetol* 2015; **52**: 47-53 [PMID: 24908579 DOI: 10.1007/s00592-014-0597-8]

40 **Pflipsen MC**, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B(12) deficiency in patients with type 2 diabetes: a cross-sectional study. *J Am Board Fam Med* 2009; **22**: 528-534 [PMID: 19734399 DOI: 10.3122/jabfm.2009.05.090044]

41 **Rodríguez-Gutiérrez R**, Montes-Villarreal J, Rodríguez-Velver KV, González-Velázquez C, Salcido-Montenegro A, Elizondo-Plazas A, González-González JG. Metformin Use and Vitamin B12 Deficiency: Untangling the Association. *Am J Med Sci* 2017; **354**: 165-171 [PMID: 28864375 DOI: 10.1016/j.amjms.2017.04.010]

42 **Berchtold P**, Bolli P, Arbenz U, Keiser G. [Disturbance of intestinal absorption following metformin therapy (observations on the mode of action of biguanides]. *Diabetologia* 1969; **5**: 405-412 [PMID: 5372893 DOI: 10.1007/BF00427979]

43 **Tomkin GH**, Hadden DR, Weaver JA, Montgomery DA. Vitamin-B12 status of patients on long-term metformin therapy. *Br Med J* 1971; **2**: 685-687 [PMID: 5556053 DOI: 10.1136/bmj.2.5763.685]

44 **Ting RZ**, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B(12) deficiency in patients receiving metformin. *Arch Intern Med* 2006; **166**: 1975-1979 [PMID: 17030830 DOI: 10.1001/archinte.166.18.1975]

45 **Shivaprasad C**, Gautham K, Ramdas B, Gopaldatta KS, Nishchitha K. Metformin Usage Index and assessment of vitamin B12 deficiency among metformin and non-metformin users with type 2 diabetes mellitus. *Acta Diabetol* 2020; **57**: 1073-1080 [PMID: 32266492 DOI: 10.1007/s00592-020-01526-4]

46 **American Diabetes Association**. 3. Prevention or Delay of Type 2 Diabetes: *Standards of Medical Care in Diabetes-2021*. *Diabetes Care* 2021; **44**: S34-S39 [PMID: 33298414 DOI: 10.2337/dc21-S003]

47 **Martin D**, Thaker J, Shreve M, Lamerato L, Budzynska K. Assessment of vitamin B12 deficiency and B12 screening trends for patients on metformin: a retrospective cohort case review. *BMJ Nutr Prevent Health* 2021 [DOI: 10.1136/bmjnph-2020-000193]

48 **Wile DJ**, Toth C. Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy. *Diabetes Care* 2010; **33**: 156-161 [PMID: 19846797 DOI: 10.2337/dc09-0606]

49 **Out M**, Kooy A, Lehert P, Schalkwijk CA, Stehouwer CDA. Long-term treatment with metformin in type 2 diabetes and methylmalonic acid: Post hoc analysis of a randomized controlled 4.3year trial. *J Diabetes Complications* 2018; **32**: 171-178 [PMID: 29174300 DOI: 10.1016/j.jdiacomp.2017.11.001]

50 **Carmel R**. Current concepts in cobalamin deficiency. *Annu Rev Med* 2000; **51**: 357-375 [PMID: 10774470 DOI: 10.1146/annurev.med.51.1.357]

51 **Stover PJ**. Vitamin B12 and older adults. *Curr Opin Clin Nutr Metab Care* 2010; **13**: 24-27 [PMID: 19904199 DOI: 10.1097/MCO.0b013e328333d157]

52 **Leung S**, Mattman A, Snyder F, Kassam R, Meneilly G, Nexo E. Metformin induces reductions in plasma cobalamin and haptocorrin bound cobalamin levels in elderly diabetic patients. *Clin Biochem* 2010; **43**: 759-760 [PMID: 20214894 DOI: 10.1016/j.clinbiochem.2010.02.011]

53 **Wakeman M**, Archer DT. Metformin and Micronutrient Status in Type 2 Diabetes: Does Polypharmacy Involving Acid-Suppressing Medications Affect Vitamin B12 Levels? *Diabetes Metab Syndr Obes* 2020; **13**: 2093-2108 [PMID: 32606868 DOI: 10.2147/DMSO.S237454]

54 **Long AN**, Atwell CL, Yoo W, Solomon SS. Vitamin B(12) deficiency associated with concomitant metformin and proton pump inhibitor use. *Diabetes Care* 2012; **35**: e84 [PMID: 23173145 DOI: 10.2337/dc12-0980]

55 **Hashem MM**, Esmael A, Nassar AK, El-Sherif M. The relationship between exacerbated diabetic peripheral neuropathy and metformin treatment in type 2 diabetes mellitus. *Sci Rep* 2021; **11**: 1940 [PMID: 33479439 DOI: 10.1038/s41598-021-81631-8]

56 **Didangelos T**, Karlafti E, Kotzakioulafi E, Margariti E, Giannoulaki P, Batanis G, Tesfaye S, Kantartzis K. Vitamin B12 Supplementation in Diabetic Neuropathy: A 1-Year, Randomized, Double-Blind, Placebo-Controlled Trial. *Nutrients* 2021; **13** [PMID: 33513879 DOI: 10.3390/nu13020395]

57 **Greibe E**, Miller JW, Foutouhi SH, Green R, Nexo E. Metformin increases liver accumulation of vitamin B12 - an experimental study in rats. *Biochimie* 2013; **95**: 1062-1065 [PMID: 23402786 DOI: 10.1016/j.biochi.2013.02.002]

58 **Adams JF**, Clark JS, Ireland JT, Kesson CM, Watson WS. Malabsorption of vitamin B12 and intrinsic factor secretion during biguanide therapy. *Diabetologia* 1983; **24**: 16-18 [PMID: 6825978 DOI: 10.1007/BF00275941]

59 **Caspary WF**, Zavada I, Reimold W, Deuticke U, Emrich D, Willms B. Alteration of bile acid metabolism and vitamin-B12-absorption in diabetics on biguanides. *Diabetologia* 1977; **13**: 187-193 [PMID: 873086 DOI: 10.1007/BF01219698]

60 **Andrès E**, Noel E, Goichot B. Metformin-associated vitamin B12 deficiency. *Arch Intern Med* 2002; **162**: 2251-2252 [PMID: 12390080 DOI: 10.1001/archinte.162.19.2251-a]

61 **Bauman WA**, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diabetes Care* 2000; **23**: 1227-1231 [PMID: 10977010 DOI: 10.2337/diacare.23.9.1227]

62 **Devalia V**, Hamilton MS, Molloy AM; British Committee for Standards in Haematology. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *Br J Haematol* 2014; **166**: 496-513 [PMID: 24942828 DOI: 10.1111/bjh.12959]

63 **Nederstigt C**, Uitbeijerse BS, Janssen LGM, Corssmit EPM, de Koning EJP, Dekkers OM. Associated auto-immune disease in type 1 diabetes patients: a systematic review and meta-analysis. *Eur J Endocrinol* 2019; **180**: 135-144 [PMID: 30508413 DOI: 10.1530/EJE-18-0515]

64 **Perros P**, Singh RK, Ludlam CA, Frier BM. Prevalence of pernicious anaemia in patients with Type

1 diabetes mellitus and autoimmune thyroid disease. *Diabet Med* 2000; **17**: 749-751 [PMID: 11110510 DOI: 10.1046/j.1464-5491.2000.00373.x]

65 **Bor MV**, von Castel-Roberts KM, Kauwell GP, Stabler SP, Allen RH, Maneval DR, Bailey LB, Nexo E. Daily intake of 4 to 7 microg dietary vitamin B-12 is associated with steady concentrations of vitamin B-12-related biomarkers in a healthy young population. *Am J Clin Nutr* 2010; **91**: 571-577 [PMID: 20071646 DOI: 10.3945/ajcn.2009.28082]

66 **Tucker KL**, Rich S, Rosenberg I, Jacques P, Dallal G, Wilson PW, Selhub J. Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. *Am J Clin Nutr* 2000; **71**: 514-522 [PMID: 10648266 DOI: 10.1093/ajcn/71.2.514]

67 **Paul C**, Brady DM. Comparative Bioavailability and Utilization of Particular Forms of B_{12} Supplements With Potential to Mitigate B_{12}-related Genetic Polymorphisms. *Integr Med (Encinitas)* 2017; **16**: 42-49 [PMID: 28223907]

68 **Estourgie-van Burk GF**, van der Kuy PHM, de Meij TG, Benninga MA, Kneepkens CMF. Intranasal treatment of vitamin B_{12} deficiency in children. *Eur J Pediatr* 2020; **179**: 349-352 [PMID: 31758311 DOI: 10.1007/s00431-019-03519-0]

69 **Del Bo' C**, Riso P, Gardana C, Brusamolino A, Battezzati A, Ciappellano S. Effect of two different sublingual dosages of vitamin B_{12} on cobalamin nutritional status in vegans and vegetarians with a marginal deficiency: A randomized controlled trial. *Clin Nutr* 2019; **38**: 575-583 [PMID: 29499976 DOI: 10.1016/j.clnu.2018.02.008]

70 **Berlin H**, Berlin R, Brante G. Oral treatment of pernicious anemia with high doses of vitamin B12 without intrinsic factor. *Acta Med Scand* 1968; **184**: 247-258 [PMID: 5751528 DOI: 10.1111/j.0954-6820.1968.tb02452.x]

71 **Hunt A**, Harrington D, Robinson S. Vitamin B12 deficiency. *BMJ* 2014; **349**: g5226 [PMID: 25189324 DOI: 10.1136/bmj.g5226]

72 **Saeedi P**, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843 [PMID: 31518657 DOI: 10.1016/j.diabres.2019.107843]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 932-938

DOI: 10.4239/wjd.v12.i7.932

ISSN 1948-9358 (online)

OPINION REVIEW

Exploring new treatment options for polycystic ovary syndrome: Review of a novel antidiabetic agent SGLT2 inhibitor

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Author contributions: Marinkovic-Radosevic J and Cigrovski Berkovic M made contributions to the conception and design of the study, drafted, and revised the manuscript critically; Kruezi E, Bilic-Curcic I, and Mrzljak A collected data, drafted and wrote the manuscript; All authors read and approved the final manuscript.

Conflict-of-interest statement: The authors declare no conflicts of interest.

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Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age associated with long-term metabolic and cardiovascular consequences. A plethora of symptoms and their severity differentiate on an individual level, giving the syndrome numerous phenotypes. Due to menstrual cycle abnormalities, women suffer from irregular menstrual bleeding, difficulty in conception, and infertility. Furthermore, the risk of pregnancy complications such as gestational diabetes mellitus, hypertensive disorders of pregnancy, and preterm birth are higher in women with PCOS than in the general population. Often, women with PCOS have comorbidities such as dyslipidemia, obesity, glucose intolerance or diabetes type 2, non-alcoholic fatty liver disease, and metabolic syndrome, which all influence the treatment plan. Historic insulin-sensitizing agents, although good for some of the metabolic derangements, do not offer long-term cardiovascular benefits; therefore, new treatment options are of

Manuscript source: Invited manuscript

Specialty type: Medicine, general and internal

Country/Territory of origin: Croatia

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 13, 2021
Peer-review started: March 13, 2021
First decision: March 30, 2021
Revised: March 30, 2021
Accepted: June 22, 2021
Article in press: June 22, 2021
Published online: July 15, 2021

P-Reviewer: Morozov S
S-Editor: Liu M
L-Editor: Filipodia
P-Editor: Wang LL

paramount importance. Sodium-glucose co-transporter-2 (SGLT-2) inhibitors, a new class of antidiabetic agents with beneficial cardiovascular, bodyweight, and antihyperglycemic effects, although not approved for the treatment of PCOS, might be an attractive therapeutic addition in the PCOS armamentarium. Namely, recent studies with SGLT-2 inhibitors showed promising improvements in anthropometric parameters and body composition in patients with PCOS. It is important to further explore the SGLT-2 inhibitors potential as an early therapeutic option because of the PCOS-related risk of metabolic, reproductive, and psychological consequences.

Key Words: Polycystic ovary syndrome; Sodium-glucose co-transporter-2 inhibitors; Metabolic risk; Cardiovascular risk; Metabolic syndrome; Insulin resistance; Obesity; Type 2 diabetes mellitus; Dyslipidemia

Core Tip: Sodium-glucose co-transporter-2 (SGLT-2) inhibitors are a new class of antidiabetic agents with beneficial cardiovascular, bodyweight, and antihyperglycemic effects. Although not approved for the treatment of polycystic ovary syndrome (PCOS), they might be an attractive therapeutic addition for the related metabolic, reproductive, and psychological consequences. Recent studies with SGLT-2 inhibitors in PCOS patients showed promising improvements in anthropometric parameters and body composition. Thus, it is important to explore the SGLT-2 inhibitors potential as an early therapeutic option in PCOS due to its high cardiometabolic risk.

Citation: Marinkovic-Radosevic J, Cigrovski Berkovic M, Kruezi E, Bilic-Curcic I, Mrzljak A. Exploring new treatment options for polycystic ovary syndrome: Review of a novel antidiabetic agent SGLT2 inhibitor. *World J Diabetes* 2021; 12(7): 932-938
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/932.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.932

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex condition defined by metabolic, fertility, and psychological consequences, with a prevalence of up to 20% among females of reproductive age[1]. It is diagnosed according to Rotterdam criteria (2 of the following): Oligo- or anovulation, clinical and/or biochemical hyperandrogenemia, or polycystic ovarian morphology on ultrasound[2]. Underlying pathologic processes create a broad spectrum of clinical and laboratory abnormalities. The underlying mechanisms are still inconclusive, however certain genetic traits[3-5], altered gonadotropin secretion[6,7], faulty ovarian follicle maturation[8], and insulin resistance (IR)[9] are considered the most important etiological factors. IR leads to hyperinsulinemia which precipitates hyperandrogenaemia by stimulating ovarian androgen secretion and inhibition of hepatic sex hormone-binding globulin (SHBG) production[10].

A plethora of symptoms and their severity differentiate on an individual level, giving the syndrome numerous phenotypes. Due to menstrual cycle abnormalities, women suffer from irregular menstrual bleeding, difficulty in conception, and infertility. Furthermore, the risk of pregnancy complications such as gestational diabetes mellitus, hypertensive disorders of pregnancy, and preterm birth are higher in women with PCOS than in the general population[11,12].

Although cardiometabolic risk factors are not part of the PCOS diagnostic criteria, they impact the treatment and prognosis[13]. Metabolic issues related to PCOS increase the risk for long-term consequences such as dyslipidemia, obesity, glucose intolerance[14], diabetes type 2[15-17], low-grade chronic inflammation[18], non-alcoholic fatty liver disease[19,20] and metabolic syndrome[21,22]. Consequently, all women with PCOS should be assessed for cardiovascular risk factors and global cardiovascular disease risk[2].

Treatment goals for PCOS include diminishing clinical hyperandrogenism, managing menstrual dysfunction, preventing endometrial hyperplasia and carcinoma, accomplishing ovulation in pursuit of pregnancy, and regulating metabolic issues in the long term. Lifestyle changes and weight loss are the cornerstones of treatment[23]. Oral contraceptives (OCs) are the first line of PCOS pharmacotherapy due to their effect on hyperandrogenism, menstrual irregularity, and endometrial carcinoma prevention[2,24]. In cases of prevalent hyperandrogenism despite OCs, antiandrogens can be added. When pursuing pregnancy, ovulation induction should be considered with clomiphene citrate, letrozole, and, rarely, gonadotropins[25]. If weight loss and ovulation induction are not successful, the next step is *in vitro* fertilization.

In the case of metabolic derangements, insulin-sensitizing agents, primarily metformin and thiazolidinediones, are widely used as an alternative or add-on to OCs [26,27]. Studies of newer glucose-lowering agents, such as glucagon-like peptide-1 receptor analogs (GLP-1RA) used for the treatment of obese women with PCOS, revealed a reduction of body weight, increase in menstrual frequency, and improvement of hyperandrogenemia and metabolic derangements even more effectively than metformin[28,29]. The down-side of the mentioned therapy might be a subcutaneous way of application.

PCOS AND CARDIOMETABOLIC RISK

One of the most important pathophysiological processes involved in PCOS development includes IR. The prevalence of IR in PCOS is high: it affects 75% of lean and 95% overweight women[30]. IR represents a link towards increased cardiometabolic risk leading to conditions such as hypertension, glucose intolerance or diabetes, dyslipidemia, and obesity[9,31].

Up to 70% of PCOS women demonstrate IR, glucose intolerance, and overt diabetes [32]. An American study on Women's Health Across the Nation showed a higher prevalence of impaired glucose tolerance (IGT) in PCOS (25%) compared to controls (9.2%)[33]. Moreover, a recent meta-analysis in women with PCOS demonstrated an increased prevalence of type 2 diabetes (T2DM) (odds ratio = 2.87, 95%CI: 1.44-5.72) [34]. Interestingly, 15%-36% of all T2DM diagnosed in women, irrespective of age, is found in association with PCOS. Women with PCOS often exhibit insulin secretory impairment, which accelerates the progression from IGT to T2DM 5 to 10-fold compared to the non-PCOS population, leading to prevalence rates of T2DM 5 to 7-fold higher than those reported in population-based studies of women aged 20-44 years[15,35].

In addition, dyslipidemia occurs in up to 70% of women with PCOS, most commonly characterized by high triglyceride, increased small dense LDL-C levels, and low HDL-C levels[36]. Obesity is also highly prevalent in PCOS; up to 60% of women with PCOS have body mass index (BMI) in the overweight or obesity range, which predisposes them to IR, gonadotropin secretion disturbances, hyperandrogenemia, and low SHBG secretion[37-39]. Consequently, metabolic syndrome is commonly found in women with PCOS (prevalence of 33%-47% in the United States, and 8%-25% in other countries)[36].

According to the recent guidelines, all women with PCOS should be screened for cardiovascular risk factors. Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society recommends categorizing PCOS related cardiovascular disease (CVD) risk as patients at risk (PCOS patients with obesity, cigarette smoking, hypertension, dyslipidemia, subclinical vascular disease, IGT, family history of premature CVD) or high risk (PCOS patients with metabolic syndrome, diabetes mellitus or overt vascular/renal disease)[36].

ROLE OF SGLT-2 INHIBITORS IN PCOS

Multiple metabolic disorders are well recognized among PCOS patients, so assessing the glycemic status is essential. If IGT is detected, lifestyle interventions together with insulin-sensitizing agents such as metformin and thiazolidinedione can be added to improve insulin sensitivity[31]. There are no dedicated metformin studies to confirm its effects on superior BMI reduction compared to placebo or decrease in central adiposity, a good marker for metabolic syndrome[26,27]. Incretins, primarily GLP-1RAs, have the potential to overcome metabolic derangements of PCOS and adding cardiovascular benefits. However, their use is limited by the need for subcutaneous

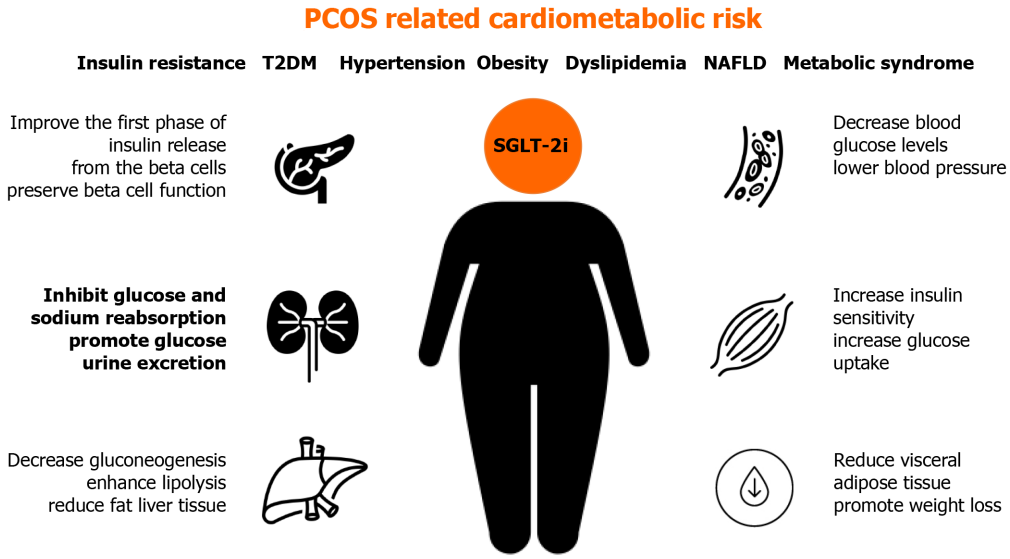

Figure 1 Potential benefits of sodium-glucose co-transporter-2 inhibitors in treatment of different metabolic and cardiovascular features of polycystic ovary syndrome. NAFLD: Non-alcoholic fatty liver disease; PCOS: Polycystic ovary syndrome; SGLT-2i: Sodium-glucose co-transporter-2 inhibitors; T2DM: Type 2 diabetes.

application, while dipeptidyl peptidase-4 inhibitors, oral incretin therapy, lack the evidence of cardiovascular protection in recently published dedicated CVOTs[40]. Therefore, there is still a considerable demand for safe and effective therapeutic agents, offering solutions against PCOS's metabolic dysregulation.

Although SGLT-2 inhibitors are not approved for PCOS treatment, this class of antidiabetic drugs could be useful for PCOS patients due to beneficial glycemic and cardiovascular effects, which are often an issue in women affected by PCOS[41] (Figure 1).

SGLT receptors are not found on ovaries, but their inhibition can indirectly improve metabolic status disrupted in certain PCOS patients. The role of SGLT-2 inhibitors in the treatment of PCOS is not yet well studied. Their mode of action can contribute to several pathophysiologic disorders in PCOS, including previously mentioned IR, hypertension, obesity, and dyslipidemia. By binding to SGLT-2 receptors in the proximal convoluted tubule of the kidney, gliflozins inhibit glucose and sodium reabsorption, causing a decrease in blood glucose levels, glucosuria, and natriuresis, which contributes to lowering blood pressure. Gliflozins promote glucose urine excretion by 60-80 g per day (approximately 240-320 kcal/d), promoting weight loss by approximately 1.7 kg. The action of SGLT-2 inhibitors does not depend on insulin secretion, beta-cell function, or IR[42,43].

SGLT-2 inhibitors achieve a further reduction in blood glucose levels by increasing insulin sensitivity, increasing glucose uptake in the muscle, decreasing gluconeogenesis in the liver, and improving the first phase of insulin release from the pancreatic beta-cells.

All mentioned processes improve metabolic profiles in diabetic patients, including lipid levels and serum uric acid levels, which could also be beneficial for PCOS patients[44]. Research also suggests the role of SGLT-2 inhibitors in preserving beta-cell function by indirectly reducing insulin secretion and promoting glucagon secretion. The latter plays a role in enhancing lipolysis and reducing the liver and visceral adipose tissue[45].

Besides the expected effect of SGLT-2 inhibitors on glycemic control, they are also shown to be cardioprotective[46], which is an important benefit regarding an increased risk of cardiovascular disease in PCOS.

So far, only one randomized controlled trial compared the effects of empagliflozin (25 mg) *vs* metformin (1500 mg) on anthropometric and body composition, hormonal and metabolic parameters in 39 women with PCOS. Group treated with empagliflozin showed beneficial effects on weight, BMI, waist circumference and hip circumference, and total body fat in overweight and obese women with PCOS compared to metformin, but no differences were seen in hormonal and metabolic parameters, including IR and androgen levels[47]. The study comparing the effects of another SGLT-2 inhibitor, canagliflozin *vs* metformin in PCOS, is still underway (Clinical Trial Gov Identifier: NCT04700839).

CONCLUSION

Until more research confirms the positive metabolic effect of SGLT-2 inhibitors in PCOS patients, the mainstream treatment option will be lifestyle intervention, metformin, and oral contraceptive pills[24]. However, this treatment strategy does not successfully address long-term cardiometabolic consequences of PCOS[48], so SGLT-2 inhibitors, due to their mode of action, emerge as a potential new treatment option for PCOS.

ACKNOWLEDGEMENTS

The authors thank Antonije and Hrvojka Dolić for graphic design support.

REFERENCES

1 **Bozdag G**, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod* 2016; **31**: 2841-2855 [PMID: 27664216 DOI: 10.1093/humrep/dew218]
2 **Teede HJ**, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril* 2018; **110**: 364-379 [PMID: 30033227 DOI: 10.1016/j.fertnstert.2018.05.004]
3 **Vink JM**, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch twin-family study. *J Clin Endocrinol Metab* 2006; **91**: 2100-2104 [PMID: 16219714 DOI: 10.1210/jc.2005-1494]
4 **Kahsar-Miller MD**, Nixon C, Boots LR, Go RC, Azziz R. Prevalence of polycystic ovary syndrome (PCOS) in first-degree relatives of patients with PCOS. *Fertil Steril* 2001; **75**: 53-58 [PMID: 11163816 DOI: 10.1016/s0015-0282(00)01662-9]
5 **Legro RS**, Driscoll D, Strauss JF 3rd, Fox J, Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *Proc Natl Acad Sci USA* 1998; **95**: 14956-14960 [PMID: 9843997 DOI: 10.1073/pnas.95.25.14956]
6 **Balen AH**. Hypersecretion of luteinizing hormone and the polycystic ovary syndrome. *Hum Reprod* 1993; **8** Suppl 2: 123-128 [PMID: 8276945 DOI: 10.1093/humrep/8.suppl_2.123]
7 **Jakimiuk AJ**, Weitsman SR, Navab A, Magoffin DA. Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries. *J Clin Endocrinol Metab* 2001; **86**: 1318-1323 [PMID: 11238527 DOI: 10.1210/jcem.86.3.7318]
8 **Fauser BC**, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev* 1997; **18**: 71-106 [PMID: 9034787 DOI: 10.1210/edrv.18.1.0290]
9 **Osibogun O**, Ogunmoroti O, Michos ED. Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention. *Trends Cardiovasc Med* 2020; **30**: 399-404 [PMID: 31519403 DOI: 10.1016/j.tcm.2019.08.010]
10 **Sanchez-Garrido MA**, Tena-Sempere M. Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies. *Mol Metab* 2020; **35**: 100937 [PMID: 32244180 DOI: 10.1016/j.molmet.2020.01.001]
11 **Qin JZ**, Pang LH, Li MJ, Fan XJ, Huang RD, Chen HY. Obstetric complications in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Reprod Biol Endocrinol* 2013; **11**: 56 [PMID: 23800002 DOI: 10.1186/1477-7827-11-56]
12 **Sha T**, Wang X, Cheng W, Yan Y. A meta-analysis of pregnancy-related outcomes and complications in women with polycystic ovary syndrome undergoing IVF. *Reprod Biomed Online* 2019; **39**: 281-293 [PMID: 31255606 DOI: 10.1016/j.rbmo.2019.03.203]
13 **Oliver-Williams C**, Vassard D, Pinborg A, Schmidt L. Risk of cardiovascular disease for women with polycystic ovary syndrome: results from a national Danish registry cohort study. *Eur J Prev Cardiol* 2020; 2047487320939674 [PMID: 32741211 DOI: 10.1177/2047487320939674]
14 **Legro RS**, Gnatuk CL, Kunselman AR, Dunaif A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. *J Clin Endocrinol Metab* 2005; **90**: 3236-3242 [PMID: 15797965 DOI: 10.1210/jc.2004-1843]
15 **Ehrmann DA**, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care* 1999; **22**: 141-146 [PMID: 10333916 DOI: 10.2337/diacare.22.1.141]
16 **Ehrmann DA**, Kasza K, Azziz R, Legro RS, Ghazzi MN; PCOS/Troglitazone Study Group. Effects of race and family history of type 2 diabetes on metabolic status of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2005; **90**: 66-71 [PMID: 15507516 DOI: 10.1210/jc.2004-0229]
17 **Lo JC**, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS. Epidemiology and adverse

cardiovascular risk profile of diagnosed polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; **91**: 1357-1363 [PMID: 16434451 DOI: 10.1210/jc.2005-2430]

18 **Spritzer PM**, Lecke SB, Satler F, Morsch DM. Adipose tissue dysfunction, adipokines, and low-grade chronic inflammation in polycystic ovary syndrome. *Reproduction* 2015; **149**: R219-R227 [PMID: 25628442 DOI: 10.1530/REP-14-0435]

19 **Setji TL**, Holland ND, Sanders LL, Pereira KC, Diehl AM, Brown AJ. Nonalcoholic steatohepatitis and nonalcoholic Fatty liver disease in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; **91**: 1741-1747 [PMID: 16492691 DOI: 10.1210/jc.2005-2774]

20 **Rocha ALL**, Faria LC, Guimarães TCM, Moreira GV, Cândido AL, Couto CA, Reis FM. Non-alcoholic fatty liver disease in women with polycystic ovary syndrome: systematic review and meta-analysis. *J Endocrinol Invest* 2017; **40**: 1279-1288 [PMID: 28612285 DOI: 10.1007/s40618-017-0708-9]

21 **Ford ES**, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among u.s. Adults. *Diabetes Care* 2004; **27**: 2444-2449 [PMID: 15451914 DOI: 10.2337/diacare.27.10.2444]

22 **Apridonidze T**, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2005; **90**: 1929-1935 [PMID: 15623819 DOI: 10.1210/jc.2004-1045]

23 **Crosignani PG**, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. *Hum Reprod* 2003; **18**: 1928-1932 [PMID: 12923151 DOI: 10.1093/humrep/deg367]

24 **Legro RS**, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK; Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2013; **98**: 4565-4592 [PMID: 24151290 DOI: 10.1210/jc.2013-2350]

25 **Legro RS**, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, Christman GM, Huang H, Yan Q, Alvero R, Haisenleder DJ, Barnhart KT, Bates GW, Usadi R, Lucidi S, Baker V, Trussell JC, Krawetz SA, Snyder P, Ohl D, Santoro N, Eisenberg E, Zhang H; NICHD Reproductive Medicine Network. Letrozole *vs* clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 2014; **371**: 119-129 [PMID: 25006718 DOI: 10.1056/NEJMoa1313517]

26 **Morley LC**, Tang T, Yasmin E, Norman RJ, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* 2017; **11**: CD003053 [PMID: 29183107 DOI: 10.1002/14651858.CD003053.pub6]

27 **Moghetti P**, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J Clin Endocrinol Metab* 2000; **85**: 139-146 [PMID: 10634377 DOI: 10.1210/jcem.85.1.6293]

28 **Lamos EM**, Malek R, Davis SN. GLP-1 receptor agonists in the treatment of polycystic ovary syndrome. *Expert Rev Clin Pharmacol* 2017; **10**: 401-408 [PMID: 28276778 DOI: 10.1080/17512433.2017.1292125]

29 **Han Y**, Li Y, He B. GLP-1 receptor agonists *vs* metformin in PCOS: a systematic review and meta-analysis. *Reprod Biomed Online* 2019; **39**: 332-342 [PMID: 31229399 DOI: 10.1016/j.rbmo.2019.04.017]

30 **Stepto NK**, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, Teede HJ. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. *Hum Reprod* 2013; **28**: 777-784 [PMID: 23315061 DOI: 10.1093/humrep/des463]

31 **Goodarzi MO**, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol* 2011; **7**: 219-231 [PMID: 21263450 DOI: 10.1038/nrendo.2010.217]

32 **Geller DH**, Pacaud D, Gordon CM, Misra M; of the Drug and Therapeutics Committee of the Pediatric Endocrine Society. State of the Art Review: Emerging Therapies: The Use of Insulin Sensitizers in the Treatment of Adolescents with Polycystic Ovary Syndrome (PCOS). *Int J Pediatr Endocrinol* 2011; **2011**: 9 [PMID: 21899727 DOI: 10.1186/1687-9856-2011-9]

33 **Polotsky AJ**, Allshouse A, Crawford SL, Harlow SD, Khalil N, Santoro N, Legro RS. Relative contributions of oligomenorrhea and hyperandrogenemia to the risk of metabolic syndrome in midlife women. *J Clin Endocrinol Metab* 2012; **97**: E868-E877 [PMID: 22466350 DOI: 10.1210/jc.2011-3357]

34 **Kakoly NS**, Khomami MB, Joham AE, Cooray SD, Misso ML, Norman RJ, Harrison CL, Ranasinha S, Teede HJ, Moran LJ. Ethnicity, obesity and the prevalence of impaired glucose tolerance and type 2 diabetes in PCOS: a systematic review and meta-regression. *Hum Reprod Update* 2018; **24**: 455-467 [PMID: 29590375 DOI: 10.1093/humupd/dmy007]

35 **Legro RS**, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab* 1999; **84**: 165-169 [PMID: 9920077 DOI: 10.1210/jcem.84.1.5393]

36 **Wild RA**, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, Lobo R, Norman RJ, Talbott E, Dumesic DA. Assessment of cardiovascular risk and prevention of

cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab* 2010; **95**: 2038-2049 [PMID: 20375205 DOI: 10.1210/jc.2009-2724]

37 **Azziz R**, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* 2004; **89**: 2745-2749 [PMID: 15181052 DOI: 10.1210/jc.2003-032046]

38 **Legro RS**. Obesity and PCOS: implications for diagnosis and treatment. *Semin Reprod Med* 2012; **30**: 496-506 [PMID: 23074008 DOI: 10.1055/s-0032-1328878]

39 **Bajuk Studen K**, Pfeifer M. Cardiometabolic risk in polycystic ovary syndrome. *Endocr Connect* 2018; **7**: R238-R251 [PMID: 29844207 DOI: 10.1530/EC-18-0129]

40 **Jensterle Sever M**, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. *Eur J Endocrinol* 2014; **170**: 451-459 [PMID: 24362411 DOI: 10.1530/EJE-13-0797]

41 **Moran LJ**, Norman RJ, Teede HJ. Metabolic risk in PCOS: phenotype and adiposity impact. *Trends Endocrinol Metab* 2015; **26**: 136-143 [PMID: 25591984 DOI: 10.1016/j.tem.2014.12.003]

42 **Satoh H**. Pleiotropic effects of SGLT2 inhibitors beyond the effect on glycemic control. *Diabetol Int* 2018; **9**: 212-214 [PMID: 30603370 DOI: 10.1007/s13340-018-0367-x]

43 **Abdalla MA**, Deshmukh H, Atkin S, Sathyapalan T. A review of therapeutic options for managing the metabolic aspects of polycystic ovary syndrome. *Ther Adv Endocrinol Metab* 2020; **11**: 2042018820938305 [PMID: 32670541 DOI: 10.1177/2042018820938305]

44 **Verma S**, McMurray JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review. *Diabetologia* 2018; **61**: 2108-2117 [PMID: 30132036 DOI: 10.1007/s00125-018-4670-7]

45 **Hallow KM**, Greasley PJ, Helmlinger G, Chu L, Heerspink HJ, Boulton DW. Evaluation of renal and cardiovascular protection mechanisms of SGLT2 inhibitors: model-based analysis of clinical data. *Am J Physiol Renal Physiol* 2018; **315**: F1295-F1306 [PMID: 30019930 DOI: 10.1152/ajprenal.00202.2018]

46 **Heerspink HJ**, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation* 2016; **134**: 752-772 [PMID: 27470878 DOI: 10.1161/CIRCULATIONAHA.116.021887]

47 **Javed Z**, Papageorgiou M, Deshmukh H, Rigby AS, Qamar U, Abbas J, Khan AY, Kilpatrick ES, Atkin SL, Sathyapalan T. Effects of empagliflozin on metabolic parameters in polycystic ovary syndrome: A randomized controlled study. *Clin Endocrinol (Oxf)* 2019; **90**: 805-813 [PMID: 30866088 DOI: 10.1111/cen.13968]

48 **Al Khalifah RA**, Florez ID, Zoratti MJ, Dennis B, Thabane L, Bassilious E. Efficacy of Treatments for Polycystic Ovarian Syndrome Management in Adolescents. *J Endocr Soc* 2021; **5**: bvaa155 [PMID: 33324861 DOI: 10.1210/jendso/bvaa155]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 939-953

DOI: 10.4239/wjd.v12.i7.939

ISSN 1948-9358 (online)

REVIEW

Role of interferons in diabetic retinopathy

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Author contributions: Zhou YD conceived and designed the study; Li BY wrote the majority of the manuscript and prepared the tables; all authors checked the references and approved the final version of the manuscript.

Supported by National Natural Science Foundation of China, No. 81800855 and No. 82070967; and Natural Science Foundation of Hunan Province, No. 2018JJ3765.

Conflict-of-interest statement: The authors declare that no competing interest exists.

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Abstract

Diabetic retinopathy (DR) is one of the major causes of visual impairment and irreversible blindness in developed regions. Aside from abnormal angiogenesis, inflammation is the most specific and might be the initiating factor of DR. As a key participant in inflammation, interferon-gamma (IFN-γ) can be detected in different parts of the eye and is responsible for the breakdown of the blood-retina barrier and activation of inflammatory cells and other cytokines, which accelerate neovascularization and neuroglial degeneration. In addition, IFN-γ is involved in other vascular complications of diabetes mellitus and angiogenesis-dependent diseases, such as diabetic nephropathy, cerebral microbleeds, and age-related macular degeneration. Traditional treatments, such as anti-vascular endothelial growth factor agents, vitrectomy, and laser photocoagulation therapy, are more effective for angiogenesis and not tolerable for every patient. Many ongoing clinical trials are exploring effective drugs that target inflammation. For instance, IFN-α acts against viruses and angiogenesis and is commonly used to treat malignant tumors. Moreover, IFN-α has been shown to contribute to alleviating the progression of DR and other ocular diseases. In this review, we emphasize the roles that IFNs play in the pathogenesis of DR and discuss potential clinical applications of IFNs in DR, such as diagnosis, prognosis, and therapeutic treatment.

Key Words: Interferons; Cytokines; Diabetic retinopathy; Interferon-alpha; Interferon-gamma; Inflammation

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 20, 2021
Peer-review started: January 20, 2021
First decision: April 6, 2021
Revised: April 15, 2021
Accepted: May 25, 2021
Article in press: May 25, 2021
Published online: July 15, 2021

P-Reviewer: Salceda R
S-Editor: Yan JP
L-Editor: Wang TQ
P-Editor: Ma YJ

Core Tip: Diabetic retinopathy (DR) is one of the microvascular complications of diabetes mellitus and seriously threatens the eyesight of the working-age population. Inflammation and inflammatory cytokines are closely related with its pathological mechanisms. Here we discuss the roles of interferons in DR, mainly from the pathogenesis and clinical applications.

Citation: Li BY, Tan W, Zou JL, He Y, Yoshida S, Jiang B, Zhou YD. Role of interferons in diabetic retinopathy. *World J Diabetes* 2021; 12(7): 939-953
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/939.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.939

INTRODUCTION

Diabetes mellitus has reached epidemic proportions globally and affects the health of populations in both developing and developed countries[1]. Diabetic retinopathy (DR) has been recognized as a neurovasculopathy of diabetes and is a leading cause of blindness in populations of 20-74 years old in many developed countries[2-4], accounting for 2.6% of blinding cases around the world[5]. Nearly 30% of diabetic patients develop into DR[6], and once the course of diabetes extends beyond 15 years, DR can occur in almost 98% of patients with type 1 diabetes (T1D) and more than 80% of patients with type 2 diabetes (T2D)[7]. Similarly, the chance of developing sight-threatening DR is higher in T1D patients (11%) than in T2D patients (3%)[8].

Clinically, DR is classified into two stages based on microvascular changes: Non-proliferative (also known as simple or background) DR (NPDR) and end-stage proliferative DR (PDR)[9]. The former is characterized by vascular tortuosity, retinal hemorrhages, microaneurysms, yellow-white hard exudations, and white cotton spots [10]. As the final phase of DR, PDR leads to severe and quick vision impairment, which is featured by aberrant neovascularization, preretinal or vitreous hemorrhages, epiretinal membrane, and tractional detachment of the retina[11,12]. To diagnose DR, fundus photography, optical coherence tomography (OCT), and fundus fluorescein angiography (FFA) are often used to measure vascular abnormalities in the retina, such as retinal blood vessel permeability and thickness[13].

Intensive control of related risk factors, such as blood glucose, blood pressure, smoking, and pregnancy, is the typical method to minimize the progression of lesions, especially in the early stage[14,15]. Anti-vascular endothelial growth factor (anti-VEGF) medications, laser photocoagulation therapy, intravitreal injections of corticosteroids, and vitreoretinal surgery are mainly used to treat DR, especially in the advanced stage[16-18]. These methods are effective in inhibiting pathological vascular proliferation, reducing diabetic macular edema (DME), and saving eyesight[19-21]. However, these methods are restricted by a short therapeutic half-life and the risk of attendant adverse reactions, such as injection site bleeding, increased intraocular pressure, endophthalmitis, loss of peripheral vision, accelerated cataract formation, and retinal detachment[22]. Consequently, detection during the early stage of DR (NPDR) is the most effective way to prevent further worsening of DR and improve treatment and prognosis.

It is well known that inflammation participates in the early phase of DR and plays an important role in DR pathogenesis. Thus, exploring the associated mechanisms of inflammation is essential to many aspects of DR, such as diagnosis, prognosis, and therapeutic treatment. In this review, we mainly summarize the crucial roles of interferons (such as IFN-γ and IFN-α) in DR pathogenesis and discuss the potential clinical applications for patients with DR.

INFLAMMATION IN DR PATHOGENESIS

To date, the mechanisms and pathogenesis of DR remain unclear. There is a consensus that DR is the result of the interactions of multiple pathways. Hyperglycemia, ischemia- and hypoxia-induced retinal microangiopathy, inflammation and leukocyte stasis, and retinal neurodegeneration are the main causes of DR[23-25], as well as oxidative stress, mitochondrial dysfunction, microRNAs, and other molecular mecha-

nisms[26-28]. Microvascular changes, such as the loss of pericytes, increased permeability, and vasoregression, lead to retinal ischemia/hypoxia through the upregulation of biological factors, such as hypoxia-inducible factor 1 (HIF-1), VEGF, and inducible nitric oxide synthase, which play crucial roles in aberrant neovascularization[29,30].

Although abnormal neovascularization is the most characteristic change in lesions, altered inflammation occurs before the development of microvascular lesions[31,32]. Leukocyte stasis, neutrophil and macrophage infiltration, complement and microglial activation, cytokine upregulation, and increased chemokine synthesis occur in the retina[11,33]. Studies have shown a reciprocal relationship between inflammation and angiogenesis[34]. To a certain extent, the onset of DR relies on the release of proinflammatory cytokines and the adhesion of leukocytes to retinal capillaries[35]. Moreover, accumulating evidence has shown that treatments to inhibit the inflammatory reaction, such as intravitreal steroids, interleukin-6 (IL-6) inhibitors, IL-6 receptor inhibitors, and integrin inhibitors, are effective in preventing the development and worsening of DR[6,23].

The upregulation of inflammatory cytokines, such as IFN-γ, IL-1β, IL-6, and IL-10, is the primary contributor to persistent low-grade inflammation[36], which can increase vascular permeability, accelerate the progression of DME, and increase angiogenic responses of endothelial cells (ECs)[37,38]. As a proinflammatory cytokine, IFN-γ can be found in different parts of the eye in DR, such as tears[39], aqueous humor[40,41], vitreous fluids[42-45], and serum[46-48], even during the early stage of DR. Moreover, clinically significant differences exist between DR and diabetes without retinopathy (DNR), or between PDR and NPDR, suggesting that IFN-γ is closely related to the occurrence and development of DR. Therefore, similar to other substances, such as hemoglobin A1c (HbA1c), VEGF, complement component C3, intercellular adhesion molecule 1, and IL-6[49-51], IFN-γ may be a potential candidate biomarker of DR and greatly contribute to diagnosis, treatment, and prognosis.

According to existing studies, IFN-γ and IFN-α are involved in DR. IFN-α induces a marked effect on not only DR, but also the pathological processes of other ocular and systematic diseases, such as conjunctival papilloma, uveitis, HIV infection, central nervous system diseases, and malignant tumors, due to its important role in innate and adaptive immunity[52-56]. Moreover, IFN-α can cause associated ocular pathophy -siological changes, such as endophthalmitis and neovascularization of the retina, when used to treat diseases of the eye or other systematic dysfunctions, such as serpiginous choroidopathy and hepatitis C[57,58].

IMMUNOLOGICAL REGULATION OF IFNS

As discussed above, IFNs might be involved in the inflammation and pathogenesis of DR. The activity of IFNs was first discovered in 1957 by Isaacs and Lindenmann[59]. IFNs are a group of glycoproteins that are synthesized and secreted by almost all cells in mammals and after stimulation by specific antigens[60]. IFNs are an endogenous family of cytokines with pleiotropic antiviral, antiproliferative, and immunomodulatory properties that play important roles in host defense mechanisms and maintaining homeostasis[54,61]. According to the cell surface receptors to which they bind, IFNs can be classified into three main families: Types I, II, and III[62]. There are various kinds of type I IFN, IFN-γ is the only type II IFN, and type III IFN consists of four molecules[63,64]. Clinically, IFNs are widely used, and each type has specific indications, such as the use of IFN-α for leukemia and melanoma and IFN-β for multiple sclerosis[65-67].

Regarding the molecular mechanisms, highly coordinated signaling events composed of viral sensors, adaptor proteins, kinases, and transcription factors can activate IFN transcription[61]. Currently, the mechanisms by which IFNs affect viruses, tumors, or other diseases are not completely understood. The Janus kinase signal transducer and activator of transcription (JAK/STAT) pathway is strongly associated with IFN signaling in viral infections[68]. Meantime, Gysemans *et al*[69] found that STAT-1 is a pivotal factor that controls the death of beta-cells and the accompanying immune-mediated diabetes. Once viral sensors such as pattern recognition receptors recognize viral proteins and nucleic acids and detect viral genes, adaptor proteins initiate a signal transduction cascade that leads to the formation of transcription factors and IFN I/III. The secreted IFNs act in an autocrine and paracrine manner. Then, the infected cells activate the JAK/STAT pathway and accelerate the expression of IFN-stimulated genes (ISGs)[70,71]. ISGs encode antiviral effectors or

molecules that are engaged in a wide array of cellular functions[72]. Additionally, ISGs regulate IFN signaling both positively and negatively[61]. For example, ISGs modulate viral replication (OAS/RNase L, ADAR, CD74, and GBP family members), viral entry (IFITM1/2/3, MOV10, and ZAP), protein translation (MB21D1, DDIT4, PKR, and MAP3K14), and viral egress (BST2/tetherin and RSAD2)[73]. Moreover, IFN-α can upregulate the expression of major histocompatibility complex (MHC) class I molecules as well as inflammation and endoplasmic reticulum stress markers in β cells and induce β cell apoptosis with IL-1β[74]. IFN-γ upregulates MHC class I and MHC class II molecules, which can increase the susceptibility of infected cells to lysis by cytotoxic T lymphocytes[75,76].

In this review, we highlight the relationship between DR and IFN-α or IFN-γ, elaborating on their key roles in DR.

IFN-γ IN DR

IFN-γ is the only type II IFN and is released by T helper 1 lymphocytes, natural killer cells, natural killer T lymphocytes, and CD8+ T cells[77]. IFN-γ can inhibit cell proliferation, modulate the activity of cytotoxic T cells, stimulate the biosynthesis of other cytokines, and is closely associated with innate and adaptive immunity[78-80]. In addition, IFN-γ and IFN-α can upregulate the expression of programmed death-ligand 1 in pancreatic β cells in the context of T1D, which may exert protective effects to resist T cell-mediated β cell apoptosis[81].

It is well documented that inflammation is a central driver of capillary occlusion and hypoxia, which can maximize the expression of VEGF[82]. IFN-γ plays a role in the etiology of DR due to its inflammatory functions. Numerous studies have improved the understanding of the relationship between IFN-γ and DR: (1) The concentration of IFN-γ is increased in tears in DR compared with those in DNR and the ratios of anti-angiogenic and angiogenic cytokines, such as IFN-γ/MCP and IFN-γ/IL-8 are decreased[39], suggesting the formation of an angiogenic environment; (2) The level of IFN-γ is higher in the aqueous humor in DR than in DNR[40,41]; and (3) The concentrations of IFN-γ in serum and vitreous fluids in DR are significantly higher than those in DNR or DM[42,45-47] (Tables 1 and 2). These studies all provide evidence that IFN-γ promotes and sustains chronic inflammation in the diabetic retina, which can result in neuro-glial degeneration, activation of inflammatory cells, vascular dysfunction, and breakdown of the blood-retina barrier (BRB)[50,83]. In addition, IFN-γ seems to be correlated with blood glucose. It has been found that IFN-γ was significantly increased in uncontrolled T2D or patients diagnosed with T2D recently but without treatment compared with patients who received effective glucose-lowering treatment[84,85]. IFN-γ is closely correlated with systolic blood pressure, platelets, mean platelet volume (MPV), and platelet distribution width (PDW)[85], which can be used to predict microvascular complications in diabetes[85,86]. The pathological changes mediated by IFN-γ not only exist in the retina, but also occur in the cornea and vitreous.

As essential parts of innate immunity, macrophages have two primary phenotypes: M1 and M2. The balance between these two phenotypes is controlled by macrophage phenotypic plasticity, inflammatory modulators, and the activity of intracellular signaling mediators and transcription factors[87]. M1 macrophages release inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), IL-1, IL-6, IL-12, type I IFN, and proteases and perform phagocytosis, while M2 macrophages perform phagocytosis and tissue repair and remodeling, and generate chemokines and anti-inflammatory cytokines such as transforming growth factor-β (TGF-β) and IL-10[76,88,89]. In DR, M1 macrophages inhibit angiogenesis and mediate inflammation, while M2 macrophages are involved in abnormal neovascularization. M1 polarization can be induced by IFN-γ[90]. Hence, IFN-γ may mediate the pathogenesis of DR by modulating the polarization of macrophages. Moreover, we know that IL-12 participates in the process of anti-angiogenesis in many diseases, such as corneal neovascularization and tumors[91,92]. Zhou *et al*[93] showed that IL-12 could mediate and inhibit pathological neovascularization in a mouse model of oxygen-induced retinopathy through the downstream molecules IP-10 (CXCL10) and MIG (CXCL9), which are mainly induced by IFN-γ. Importantly, the study demonstrated that the intravitreal injection of recombinant IL-12 did not significantly decrease the expression of VEGFA or fibroblast growth factor-2 (FGF2), which suggests that the mechanisms of IL-12 are independent of VEGFA and FGF2[93].

Table 1 Expression of interferons in samples

Source	Condition of disease	Expression
Tears	DR	(IFN-γ) ↑↑, (IFN-γ/MCP-1) ↓, (IFN-γ/IL-8) ↓[39]
	DNR	(IFN-γ) ↑[39]
Aqueous humor	PDR	(IFN-γ) ↑↑[40]
	NPDR	(IFN-γ) ↑[40]
	DR	(IFN-γ) ↑↑[41], (IFN-α) ↓↓[117,118]
	DNR	(IFN-γ) ↑[41], (IFN-α) ↓[117,118]
Vitreous fluids	DR	(IFN-γ) ↑[42]
	DM	(IFN-γ) ↑[45]
Serum	DR	(IFN-γ) ↑↑[46]
	DNR	(IFN-γ) ↑[46]
	DM	(IFN-γ) ↑[47,48]
Plasma	DM	(IFN-α) ↑, (IFN-β) ↑[113]
Retina	DR	(IFN-β) ↑[115]

DR: Diabetic retinopathy; DNR: Diabetes without retinopathy; PDR: Proliferative diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; DM: Diabetes mellitus; IFN: Interferons.

Table 2 Concentration of interferons in patient samples

IIFNs	Source	Condition of disease	Concentration
IFN-γ	Tears (multiplex bead analysis)	Controls	1463.0 ± 158.8 (pg/mL)[39]
		DNR	1612.8 ± 228.2 (pg/mL)[39]
		DR	1957.50 ± 166.1 (pg/mL)[39]
	Aqueous humor (CBA)	Controls	60.29 ± 14.17 (pg/L)[40]
		DNR	54.96 ± 16.29 (pg/L)[40]
		NPDR	114.26 ± 50.76 (pg/L)[40]
		PDR	136.36 ± 35.55 (pg/L)[40]
	Vitreous fluids (ELISA)	Controls	3.83 ± 0.80 (pg/mL)[42]
		DR	6.25 ± 0.84 (pg/mL)[42]
	Serum (ELISA)	Controls	2.9 (pg/mL)[46]
		DNR	27.8 (pg/mL)[46]
		DR	56.8 (pg/mL)[46]
IFN-α	Aqueous humor (Bio-Plex pro^{tm} magnetic color-bead-based multiplex assay)	Controls	26.2 (0-84) (pg/mL)[117]
		DNR	0 (0-20) (pg/mL)[117]
		DR	0 (0-18) (pg/mL)[117]

Data are expressed as the mean ± SEM or median (range). DNR: Diabetes without retinopathy; DR: Diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; CBA: Cytometric bead array technique; ELISA: Enzyme linked immunosorbent assay; IFN: Interferons.

In addition, IFN-γ is involved in other microcirculatory damage in diabetes. Taylor *et al*[94] showed that the proinflammatory cytokine IFN-γ and abnormal IFN-γ signaling were responsible for microglial repair of microvascular injuries and cerebral microbleeds (CMBs) in T1D through the downregulation of *P2ry12* gene expression, which decreased the accumulation and polarization of microglia. In addition, many other studies have shown that overexpressed IFN-γ was blood-derived, and entered

the brain through the injured blood-brain barrier to bind to highly-expressed IFN-γ receptors 1 and 2 on microglia[95,96]. Du *et al*[97,98] reported that IFN-γ played a protective role in the kidney in type II diabetes and could inhibit the excessive accumulation of mesangial matrix by activating the JAK2/STAT pathway, which could suppress the high glucose-induced increase in TGF-β1 and collagen IV. In addition, IFN-γ can impair renal fibrosis by inhibiting fibroblast activation and proliferation and reducing collagen synthesis[99].

Additionally, IFN-γ is involved in other neovascularization diseases, such as ischemia, clearance of malignant tumors, and age-related macular degeneration (AMD). IFN-γ plays a central role in the pathogenesis and development of AMD, which is characterized by retinal cell atrophy and choroidal neovascularization in the macula[100]. On the one hand, IFN-γ accelerates pathological progression: (1) IFN-γ selectively promotes M1 macrophage polarization through increased secretion of IFN-regulatory factors (IRFs), such as IRF-1, IRF-5, and IRF-8[101,102], and the activation of nuclear factor-κB and STAT-1[87], which can increase the secretion of inflammatory cytokines, such as IL-1β, IL-6, and TNF-α[89,103]; and (2) IFN-γ independently upregulates VEGF in retinal pigment epithelial cells through the activation of the PI-3K/Akt/mTOR/p70 S6 kinase pathway[104]. On the other hand, IFN-γ mediates protective effects in AMD: It downregulates the functions of VEGF in ECs by inhibiting necessary genes that are indispensable for VEGF bioprocessing and upregulates IL-1 receptor antagonist[105-107].

The role of IFN-γ in angiogenic diseases is inconsistent. One the one hand, IFN-γ can prevent and slow the development of vascular proliferation by increasing the proportion of M1 macrophages, and recombinant IFN-γ can reduce pathological choroidal neovascularization in a dose-dependent manner[108]. On the other hand, IFN-γ accelerates angiogenic process by increasing the expression of VEGF and upregulating other inflammatory cells and cytokines. Further studies are needed to investigate the mechanisms by which IFN-γ affects DR pathogenesis.

IFN-α IN DR

For many years, approaches to cure DR primarily included anti-VEGF agents, anti-inflammatory therapy, photocoagulation, vitrectomy, and controlling related risk factors[23,109]. With further research on DR, many novel discoveries of new targets and methods are constantly occurring, such as neuroprotective substances (somatostatin and brimonidine), polyphenols, the Tie-2 activator AKB 9778, small interfering RNAs (bevasiranib and PF04523655), encapsulated cell technology, and small intraocular pumps[109,110]. These new interventions can be applied to patients who are resistant to traditional therapies or have severe side effects.

As a type I IFN, IFN-α functions against infection, neoplasms, and immunity[111]. IFN-α is involved in the early stage of β cell death in T1D for its autoimmune ability [112], because IFN-α is markedly increased in the plasma of individuals with T1D, and inhibition of IFN-α/β receptor 1 and antibody against IFN-α can reduce the occurrence of T1D[113,114] (Table 1). Additionally, IFN-β is highly expressed in the retina of DR rats[115] (Table 1). Gerber *et al*[116] showed that IFN-α increased the expression of HIF-1α in a dose- and time-dependent manner by activating JAK1, tyrosine kinase 2, and IFN-stimulated gene factor 3, which inhibit the proliferation of vascular ECs. Priming with IFN-γ followed by IFN-α can enhance the magnitude and duration of HIF-1α. Among 13 human IFN-α subtypes[55], IFN-α 2 has been shown to have a therapeutic effect in a wide range of ophthalmological dysfunctions involving both the anterior and posterior segments of the eye[57] (Table 3). Moreover, it has been reported that IFN-α in the aqueous humor was more unmeasurable in diabetic patients than in non-diabetic patients, and its median level was decreased in turn among nondiabetic patients, DNR patients, and DR patients, suggesting that an imbalance in immune function may be involved in the pathogenesis of DR[117,118] (Table 2).

IFN-α 2a can suppress intraocular inflammation, possibly by helping regulatory T cells restore their inhibitory functions[119]. After subcutaneous injection of 6 million/IU IFN-α 2a 3 times/wk for an average of 10 mo, patients with PDR were found to have obvious improvements in visual acuity, decreased leakage of vessels, and regression of neovascularization after complete laser panretinal photocoagulation (PRP)[120]. Chronic drug use of recombinant IFN-α 2a for PDR presented certain clinical value because regression of capillary tufts and no new hemorrhage or neovascularization were found in patients during treatment[121]. Refractory DME was cured

Table 3 Applications of interferon-α in ocular disorders

IFN	Clinical applications
IFN-α-2a	Ocular surface diseases: Tumors (such as limbal conjunctival melanoma, squamous neoplasias, and conjunctival MALT lymphoma)[134, 137]; Mooren's ulcers[138]; herpes simplex keratitis[139]
	Uveal disease: Behcet's uveitis[131]; serpiginous choroiditis[140]; choroidal neovascularization[141]; HHV-8-associated uveitis[142]; chronic noninfectious posterior uveitis[143]
	Macular and retinal disorders: Uveitic CME; angiogenesis after PRP; refractory non-infectious inflammatory macular edema[144]
IFN-α-2b	Ocular surface diseases: Tumors (such as squamous cell carcinoma[145], melanocytic tumors[145], CIN[146], conjunctival papillomatosis [147], and MALT lymphoma[57]); LSCD[135]; vernal keratoconjunctivitis[132]
	Uveal disease: Metastatic uveal melanoma[148]; Behcet's uveitis[149]
	Macular and retinal disorders: CME caused by intraocular infection[150]; refractory diabetic macular edema[133]
IFN-α-2	Dendritic keratitis[151]

MALT: Mucosa-associated lymphoid tissue; HHV-8: Human herpes virus 8; CME: Cystoid macular edema; PRP: Panretinal photocoagulation; CIN: Conjunctival and corneal intraepithelial neoplasia; LSCD: Limbal stem cell deficiency; IFN: Interferons.

by IFN-α 2a at a dose of 1 million IU/mL 3 times/wk through posterior subtenon injections, and IFN-α 2a could be effective in reducing central macular thickness and improving visual acuity[122]. There are few cases of clinical use of IFN-α 2a in the treatment of DR, thus criterion of when and how to implement and evaluate the therapeutic effect of IFN-α 2a is not unified now, which needs further exploration. Based on clinical experience, clinicians can choose appropriate intervals to review the progression of the disease, such as glucose metabolism index, vision, visual field, neovascularization, and fundus examination. There is hardly any evidence that IFN-α 2b is able to treat DR. Moreover, the susceptibility to and risk for retinopathy are increased after clinicians use IFN-α as a therapeutic method for chronic hepatitis C [123,124], which is known as IFN-associated retinopathy (IAR). Recent guidelines indicate IFN-based therapy (pegylated IFN plus ribavirin) as a first-line method in treating chronic HCV patients[125]. Although this therapeutic plan is effective for most patients, it inevitably has some side effects, including adverse ophthalmological effects. Cotton-wool spots and retinal hemorrhage are the most common symptoms in patients with IAR[126], but these patients rarely exhibit decreased visual acuity and subjective symptoms[58,127]. The potential mechanism of IAR involves IFN-α-induced deposition of immune complexes in the retinal vasculature, which can lead to ischemic changes in the retina, such as occlusion of retinal capillaries, cotton-wool spots, and retinal hemorrhage[128]. Furthermore, another study explained that glucose tolerance was obviously improved by treatment with recombinant IFN-α in both nondiabetic and diabetic HCV patients[129].

IFN-α 2a is a promising treatment for DME and DR, because it can assist in preventing vision deterioration, inhibiting active neovascularization, and promoting the barrier function of ECs in the retina[64]. According to existing clinical records, the clinical effect of IFN-α 2a was only observed in patients after PRP and those with active neovascularization but not meeting the criteria of PRP treatment[120-122]. However, the therapeutic effect of IFN-α is not widely demonstrated by clinical trials and studies, and there are side effects and risks of using IFN-α, such as flu-like symptoms and increased liver enzymes[130]. In addition, the functions of IFN-α in DM and DR seem to be contradictory. On the one hand, the overexpression of IFN-α can result in the onset of T1D; on the other hand, IFN-α has the potential to treat DR and DME and help pancreatic β cells resist T cell-mediated apoptosis. Hence, more studies are needed to estimate the therapeutic effect of IFN-α.

IFN-α is also used in many other ocular disorders, such as uveitis[131], vernal keratoconjunctivitis[132], and refractory DME[133]. In cases of conjunctival melanomas and ocular surface squamous neoplasia (OSSN), intralesional IFN-α 2a injection before surgery exhibited excellent results in reducing size and vascularity, defining tumor margins, and improving prognosis[134]. The combined use of IFN-α 2b and all-trans retinoic acid is effective in patients with partial limbal stem cell deficiency (LSCD)[135]. Topical and subconjunctival administration of IFN-α 2b exhibits good effects in controlling and preventing the recurrence of OSSN, and the adverse effects of IFN-α 2b are less severe than those of 5-fluorouracil and mitomycin C[136]. To date, there have been few reports about the role and expression of other IFNs in DR, which

deserves to be further investigated.

POTENTIAL APPLICATIONS OF IFNS IN DR

So far, DR diagnosis mainly depends on clinical manifestations. Many types of technologies are utilized to observe specific pathological changes, such as fundoscopy, FFA, and OCT. Besides, some indexes, such as HbA1c and VEGF, have also been suggested for the diagnosis, treatment, or prognosis of DR.

Inflammation is thought to be an initial event in DR. IFN-γ contributes to inflammation and is involved in the early stage of DR and other microvascular lesions associated with mellitus, such as those in kidney and brain tissues. IFN-γ is tied to indices like MPV, PDW, and blood sugar, which can predict microvascular complications of DM. And a positive correlation exists between IFN-γ and HbA1c%[48,85] and inflammatory cytokines, such as IL-1β and IL-3[47]. Besides, compared with blood glucose and hemoglobin, IFN-γ has the advantage of being less susceptible to dietary changes and better reflecting inflammation of the eye. Hence, we presume that IFN-γ might be a biomarker of DR, indicating the incidence of retinopathy once diabetes has happened, the speed of progression towards PDR, and the possibility of a poor prognosis.

For early-stage patients, the control of relevant risk factors, such as blood pressure, blood sugar, smoking, and blood lipids, is recommended. Anti-VEGF is recommended as the first-line therapy for PDR, but this treatment is restricted by its short half-life, high cost, and adverse effects. Importantly, blocking VEGF is unable to attenuate disease progression completely or reverse damage to the retina, and merely delays the rate of development and alleviates symptoms, but cannot affect a permanent cure. Currently, with the expansion of medications targeting inflammatory pathogenesis, mediators of angiopoietin signaling axes, immunosuppressants, and nonsteroidal anti-inflammatory drugs, such as tocilizumab, EBI-031, and luminate, have been shown to be effective in clinical trials and need to be further verified[23,82]. Therefore, whether there is clinical effectiveness when antibodies against IFN-γ are applied locally or intralesionally is worth being further investigated. However, the findings are controversial because IFN-γ has protective effects against renal fibrosis and inhibits vascular proliferation. Consequently, this therapeutic application for DR requires additional consideration and experimental verification.

IFN-α 2 is effective in multiple ocular diseases, such as uveitis and cystoid macular edema. Clinical effectiveness has only been observed in PDR after PRP, DME, and continued neovascularization in some reports, although this factor is effective on angiogenesis and EC proliferation. And clinicians need to closely observe the progression of patients during the use of IFN-α through a series of physical examination, fundus examination, FFA, *etc*. Moreover, IFN-α enhances glucose tolerance and may improve prognosis. Hence, IFN-α may be a potential therapeutic treatment for DR in the future.

CONCLUSION

As a retinal neovascularization disease, DR is a frequent and serious microangiopathy associated with diabetes, and is the principal cause of vision loss in the working-age population[5,51]. Inflammation is a major pathological mechanism associated with the occurrence and development of DR, through the effects of many inflammatory cells and cytokines, such as IFN-γ, IL-1β, and IL-6, macrophages, and microglia. IFNs are a group of glycoproteins that are responsible for antiviral activity, inhibiting cell proliferation, and regulating immunity and malignant tumors. Every subset has corresponding functions, and they are broadly applied to malignant tumors and viral infections, such as hepatitis type C, herpes zoster, hairy cell leukemia, T cell lymphoma, and melanoma. IFN-α and IFN-γ are tightly linked with DR in the context of inflammatory pathogenesis and treatment. As a proinflammatory cytokine, IFN-γ can be detected in tears, aqueous humor, the vitreous body, and the retina, indicating that it plays a role in the breakdown of the BRB, inflammatory injuries, abnormal angiogenesis, and other processes. IFN-γ also participates in the pathogenesis of other diabetic vascular complications, such as DN and CMBs. In addition, the presence of IFN-γ results in macrophage polarization to proinflammatory M1 phenotype, which produces inflammatory cytokines, such as TNF-α, IL-8, and IL-12, and exacerbates the inflammation. Meanwhile, IFN-γ is associated with the changes and control of blood

glucose and presents a positive correlation with HbA1c%, IL-1β, and IL-3. Consequently, IFN-γ can be a prospective biomarker of DR. Based on its immunomodulatory effect, IFN-α helps to improve visual acuity, reduce uncommon vascular proliferation, and improve glucose tolerance. However, IFN-α contributes to the occurrence of T1D. IFN-α and IFN-γ also play important roles in other diseases characterized by abnormal angiogenesis, such as AMD, hepatocellular carcinoma, cervical neoplasia, and IAR by proinflammatory and immunoregulatory functions.

Therefore, we believe that IFN-γ has the potential to be a biomarker in the diagnosis and prognosis of DR, as well as to be a key regulator in treating DR. Additionally, the applications of IFN-α still need to be further explored.

REFERENCES

1 **Wong TY**, Sun J, Kawasaki R, Ruamviboonsuk P, Gupta N, Lansingh VC, Maia M, Mathenge W, Moreker S, Muqit MMK, Resnikoff S, Verdaguer J, Zhao P, Ferris F, Aiello LP, Taylor HR. Guidelines on Diabetic Eye Care: The International Council of Ophthalmology Recommendations for Screening, Follow-up, Referral, and Treatment Based on Resource Settings. *Ophthalmology* 2018; **125**: 1608-1622 [PMID: 29776671 DOI: 10.1016/j.ophtha.2018.04.007]

2 **Lee R**, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye Vis (Lond)* 2015; **2**: 17 [PMID: 26605370 DOI: 10.1186/s40662-015-0026-2]

3 **Congdon NG**, Friedman DS, Lietman T. Important causes of visual impairment in the world today. *JAMA* 2003; **290**: 2057-2060 [PMID: 14559961 DOI: 10.1001/jama.290.15.2057]

4 **Cheung N**, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet* 2010; **376**: 124-136 [PMID: 20580421 DOI: 10.1016/S0140-6736(09)62124-3]

5 **Bourne RR**, Stevens GA, White RA, Smith JL, Flaxman SR, Price H, Jonas JB, Keeffe J, Leasher J, Naidoo K, Pesudovs K, Resnikoff S, Taylor HR; Vision Loss Expert Group. Causes of vision loss worldwide, 1990-2010: a systematic analysis. *Lancet Glob Health* 2013; **1**: e339-e349 [PMID: 25104599 DOI: 10.1016/S2214-109X(13)70113-X]

6 **Dulull N**, Kwa F, Osman N, Rai U, Shaikh B, Thrimawithana TR. Recent advances in the management of diabetic retinopathy. *Drug Discov Today* 2019; **24**: 1499-1509 [PMID: 30954684 DOI: 10.1016/j.drudis.2019.03.028]

7 **Moreno A**, Lozano M, Salinas P. Diabetic retinopathy. *Nutr Hosp* 2013; **28** Suppl 2: 53-56 [PMID: 23834047 DOI: 10.3305/nh.2013.28.sup2.6714]

8 **Thomas RL**, Dunstan FD, Luzio SD, Chowdhury SR, North RV, Hale SL, Gibbins RL, Owens DR. Prevalence of diabetic retinopathy within a national diabetic retinopathy screening service. *Br J Ophthalmol* 2015; **99**: 64-68 [PMID: 25091950 DOI: 10.1136/bjophthalmol-2013-304017]

9 **Santiago AR**, Boia R, Aires ID, Ambrósio AF, Fernandes R. Sweet Stress: Coping With Vascular Dysfunction in Diabetic Retinopathy. *Front Physiol* 2018; **9**: 820 [PMID: 30057551 DOI: 10.3389/fphys.2018.00820]

10 **Sabanayagam C**, Banu R, Chee ML, Lee R, Wang YX, Tan G, Jonas JB, Lamoureux EL, Cheng CY, Klein BEK, Mitchell P, Klein R, Cheung CMG, Wong TY. Incidence and progression of diabetic retinopathy: a systematic review. *Lancet Diabetes Endocrinol* 2019; **7**: 140-149 [PMID: 30005958 DOI: 10.1016/S2213-8587(18)30128-1]

11 **Rübsam A**, Parikh S, Fort PE. Role of Inflammation in Diabetic Retinopathy. *Int J Mol Sci* 2018; **19** [PMID: 29565290 DOI: 10.3390/ijms19040942]

12 **Altmann C**, Schmidt MHH. The Role of Microglia in Diabetic Retinopathy: Inflammation, Microvasculature Defects and Neurodegeneration. *Int J Mol Sci* 2018; **19** [PMID: 29301251 DOI: 10.3390/ijms19010110]

13 **Ciulla TA**, Amador AG, Zinman B. Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies. *Diabetes Care* 2003; **26**: 2653-2664 [PMID: 12941734 DOI: 10.2337/diacare.26.9.2653]

14 **Kaštelan S**, Tomić M, Gverović Antunica A, Ljubić S, Salopek Rabatić J, Karabatić M. Body mass index: a risk factor for retinopathy in type 2 diabetic patients. *Mediators Inflamm* 2013; **2013**: 436329 [PMID: 24347825 DOI: 10.1155/2013/436329]

15 **Morrison JL**, Hodgson LA, Lim LL, Al-Qureshi S. Diabetic retinopathy in pregnancy: a review. *Clin Exp Ophthalmol* 2016; **44**: 321-334 [PMID: 27062093 DOI: 10.1111/ceo.12760]

16 **Simó R**, Hernández C. Novel approaches for treating diabetic retinopathy based on recent pathogenic evidence. *Prog Retin Eye Res* 2015; **48**: 160-180 [PMID: 25936649 DOI: 10.1016/j.preteyeres.2015.04.003]

17 **Tan GS**, Cheung N, Simó R, Cheung GC, Wong TY. Diabetic macular oedema. *Lancet Diabetes Endocrinol* 2017; **5**: 143-155 [PMID: 27496796 DOI: 10.1016/S2213-8587(16)30052-3]

18 **Gross JG**, Glassman AR, Liu D, Sun JK, Antoszyk AN, Baker CW, Bressler NM, Elman MJ, Ferris FL 3rd, Gardner TW, Jampol LM, Martin DF, Melia M, Stockdale CR, Beck RW; Diabetic Retinopathy Clinical Research Network. Five-Year Outcomes of Panretinal Photocoagulation vs Intravitreous Ranibizumab for Proliferative Diabetic Retinopathy: A Randomized Clinical Trial. *JAMA Ophthalmol* 2018; **136**: 1138-1148 [PMID: 30043039 DOI:

10.1001/jamaophthalmol.2018.3255]
19 **Das A**, McGuire PG, Rangasamy S. Diabetic Macular Edema: Pathophysiology and Novel Therapeutic Targets. *Ophthalmology* 2015; **122**: 1375-1394 [PMID: 25935789 DOI: 10.1016/j.ophtha.2015.03.024]
20 **Campochiaro PA**, Aiello LP, Rosenfeld PJ. Anti-Vascular Endothelial Growth Factor Agents in the Treatment of Retinal Disease: From Bench to Bedside. *Ophthalmology* 2016; **123**: S78-S88 [PMID: 27664289 DOI: 10.1016/j.ophtha.2016.04.056]
21 **Baker CW**, Glassman AR, Beaulieu WT, Antoszyk AN, Browning DJ, Chalam KV, Grover S, Jampol LM, Jhaveri CD, Melia M, Stockdale CR, Martin DF, Sun JK; DRCR Retina Network. Effect of Initial Management With Aflibercept vs Laser Photocoagulation vs Observation on Vision Loss Among Patients With Diabetic Macular Edema Involving the Center of the Macula and Good Visual Acuity: A Randomized Clinical Trial. *JAMA* 2019; **321**: 1880-1894 [PMID: 31037289 DOI: 10.1001/jama.2019.5790]
22 **Semeraro F**, Morescalchi F, Cancarini A, Russo A, Rezzola S, Costagliola C. Diabetic retinopathy, a vascular and inflammatory disease: Therapeutic implications. *Diabetes Metab* 2019; **45**: 517-527 [PMID: 31005756 DOI: 10.1016/j.diabet.2019.04.002]
23 **Wang W**, Lo ACY. Diabetic Retinopathy: Pathophysiology and Treatments. *Int J Mol Sci* 2018; **19** [PMID: 29925789 DOI: 10.3390/ijms19061816]
24 **Simó R**, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia* 2018; **61**: 1902-1912 [PMID: 30030554 DOI: 10.1007/s00125-018-4692-1]
25 **Hammes HP**. Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond. *Diabetologia* 2018; **61**: 29-38 [PMID: 28942458 DOI: 10.1007/s00125-017-4435-8]
26 **Wu MY**, Yiang GT, Lai TT, Li CJ. The Oxidative Stress and Mitochondrial Dysfunction during the Pathogenesis of Diabetic Retinopathy. *Oxid Med Cell Longev* 2018; **2018**: 3420187 [PMID: 30254714 DOI: 10.1155/2018/3420187]
27 **Satari M**, Aghadavod E, Mirhosseini N, Asemi Z. The effects of microRNAs in activating neovascularization pathways in diabetic retinopathy. *J Cell Biochem* 2019; **120**: 9514-9521 [PMID: 30556195 DOI: 10.1002/jcb.28227]
28 **Brownlee M**. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615-1625 [PMID: 15919781 DOI: 10.2337/diabetes.54.6.1615]
29 **Li HY**, Yuan Y, Fu YH, Wang Y, Gao XY. Hypoxia-inducible factor-1α: A promising therapeutic target for vasculopathy in diabetic retinopathy. *Pharmacol Res* 2020; **159**: 104924 [PMID: 32464323 DOI: 10.1016/j.phrs.2020.104924]
30 **Othman R**, Vaucher E, Couture R. Bradykinin Type 1 Receptor - Inducible Nitric Oxide Synthase: A New Axis Implicated in Diabetic Retinopathy. *Front Pharmacol* 2019; **10**: 300 [PMID: 30983997 DOI: 10.3389/fphar.2019.00300]
31 **Mesquida M**, Drawnel F, Fauser S. The role of inflammation in diabetic eye disease. *Semin Immunopathol* 2019; **41**: 427-445 [PMID: 31175392 DOI: 10.1007/s00281-019-00750-7]
32 **Rivera JC**, Dabouz R, Noueihed B, Omri S, Tahiri H, Chemtob S. Ischemic Retinopathies: Oxidative Stress and Inflammation. *Oxid Med Cell Longev* 2017; **2017**: 3940241 [PMID: 29410732 DOI: 10.1155/2017/3940241]
33 **Forrester JV**, Kuffova L, Delibegovic M. The Role of Inflammation in Diabetic Retinopathy. *Front Immunol* 2020; **11**: 583687 [PMID: 33240272 DOI: 10.3389/fimmu.2020.583687]
34 **Capitão M**, Soares R. Angiogenesis and Inflammation Crosstalk in Diabetic Retinopathy. *J Cell Biochem* 2016; **117**: 2443-2453 [PMID: 27128219 DOI: 10.1002/jcb.25575]
35 **Aouiss A**, Anka Idrissi D, Kabine M, Zaid Y. Update of inflammatory proliferative retinopathy: Ischemia, hypoxia and angiogenesis. *Curr Res Transl Med* 2019; **67**: 62-71 [PMID: 30685380 DOI: 10.1016/j.retram.2019.01.005]
36 **Adamis AP**, Berman AJ. Immunological mechanisms in the pathogenesis of diabetic retinopathy. *Semin Immunopathol* 2008; **30**: 65-84 [PMID: 18340447 DOI: 10.1007/s00281-008-0111-x]
37 **Tang J**, Kern TS. Inflammation in diabetic retinopathy. *Prog Retin Eye Res* 2011; **30**: 343-358 [PMID: 21635964 DOI: 10.1016/j.preteyeres.2011.05.002]
38 **Rangasamy S**, McGuire PG, Das A. Diabetic retinopathy and inflammation: novel therapeutic targets. *Middle East Afr J Ophthalmol* 2012; **19**: 52-59 [PMID: 22346115 DOI: 10.4103/0974-9233.92116]
39 **Liu J**, Shi B, He S, Yao X, Willcox MD, Zhao Z. Changes to tear cytokines of type 2 diabetic patients with or without retinopathy. *Mol Vis* 2010; **16**: 2931-2938 [PMID: 21203348 DOI: 10.1017/S0952523810000453]
40 **Wu H**, Hwang DK, Song X, Tao Y. Association between Aqueous Cytokines and Diabetic Retinopathy Stage. *J Ophthalmol* 2017; **2017**: 9402198 [PMID: 28680705 DOI: 10.1155/2017/9402198]
41 **Vujosevic S**, Micera A, Bini S, Berton M, Esposito G, Midena E. Proteome analysis of retinal glia cells-related inflammatory cytokines in the aqueous humour of diabetic patients. *Acta Ophthalmol* 2016; **94**: 56-64 [PMID: 26268591 DOI: 10.1111/aos.12812]
42 **Tsai T**, Kuehn S, Tsiampalis N, Vu MK, Kakkassery V, Stute G, Dick HB, Joachim SC. Anti-inflammatory cytokine and angiogenic factors levels in vitreous samples of diabetic retinopathy patients. *PLoS One* 2018; **13**: e0194603 [PMID: 29584759 DOI: 10.1371/journal.pone.0194603]
43 **Takeuchi M**, Sato T, Sakurai Y, Taguchi M, Harimoto K, Karasawa Y, Ito M. Association between aqueous humor and vitreous fluid levels of Th17 cell-related cytokines in patients with proliferative

diabetic retinopathy. *PLoS One* 2017; **12**: e0178230 [PMID: 28558009 DOI: 10.1371/journal.pone.0178230]
44 **Suzuki Y**, Nakazawa M, Suzuki K, Yamazaki H, Miyagawa Y. Expression profiles of cytokines and chemokines in vitreous fluid in diabetic retinopathy and central retinal vein occlusion. *Jpn J Ophthalmol* 2011; **55**: 256-263 [PMID: 21538000 DOI: 10.1007/s10384-011-0004-8]
45 **Özay Y**, Ozek D, Yıldırım F, Yıldırım Z. The effect of diabetes on vitreous levels of adiponectin and inflammatory cytokines in experimental rat model. *Adv Clin Exp Med* 2020; **29**: 449-452 [PMID: 32343888 DOI: 10.17219/acem/115004]
46 **Cvitkovic K**, Sesar A, Sesar I, Pusic-Sesar A, Pejic R, Kelava T, Sucur A, Cavar I. Concentrations of Selected Cytokines and Vascular Endothelial Growth Factor in Aqueous Humor and Serum of Diabetic Patients. *Semin Ophthalmol* 2020; **35**: 126-133 [PMID: 32310727 DOI: 10.1080/08820538.2020.1755320]
47 **Amin K**, Qadr SH, Hassan Hussein R, Ali KM, Rahman HS. Levels of cytokines and GADA in type I and II diabetic patients. *Prim Care Diabetes* 2020; **14**: 61-67 [PMID: 31014937 DOI: 10.1016/j.pcd.2019.03.008]
48 **Hasan Z**, Irfan M, Masood Q, Ahmed O, Moosajee US, Rao S, Salahuddin N. Raised levels of IFN-gamma and IL-13 are associated with pre-diabetes amongst newly diagnosed patients with Tuberculosis. *J Pak Med Assoc* 2019; **69**: 468-473 [PMID: 31000846]
49 **Kaštelan S**, Orešković I, Bišćan F, Kaštelan H, Gverović Antunica A. Inflammatory and angiogenic biomarkers in diabetic retinopathy. *Biochem Med (Zagreb)* 2020; **30**: 030502 [PMID: 32774120 DOI: 10.11613/BM.2020.030502]
50 **Youngblood H**, Robinson R, Sharma A, Sharma S. Proteomic Biomarkers of Retinal Inflammation in Diabetic Retinopathy. *Int J Mol Sci* 2019; **20** [PMID: 31557880 DOI: 10.3390/ijms20194755]
51 **Cunha-Vaz J**, Ribeiro L, Lobo C. Phenotypes and biomarkers of diabetic retinopathy. *Prog Retin Eye Res* 2014; **41**: 90-111 [PMID: 24680929 DOI: 10.1016/j.preteyeres.2014.03.003]
52 **Tseng SH**. Conjunctival papilloma. *Ophthalmology* 2009; **116**: 1013-1013. e1 [PMID: 19410961 DOI: 10.1016/j.ophtha.2008.12.035]
53 **Plskova J**, Greiner K, Forrester JV. Interferon-alpha as an effective treatment for noninfectious posterior uveitis and panuveitis. *Am J Ophthalmol* 2007; **144**: 55-61 [PMID: 17601428 DOI: 10.1016/j.ajo.2007.03.050]
54 **Reyes-Vázquez C**, Prieto-Gómez B, Dafny N. Interferon modulates central nervous system function. *Brain Res* 2012; **1442**: 76-89 [PMID: 22322149 DOI: 10.1016/j.brainres.2011.09.061]
55 **Sutter K**, Dickow J, Dittmer U. Interferon α subtypes in HIV infection. *Cytokine Growth Factor Rev* 2018; **40**: 13-18 [PMID: 29475588 DOI: 10.1016/j.cytogfr.2018.02.002]
56 **Borden EC**. Interferons α and β in cancer: therapeutic opportunities from new insights. *Nat Rev Drug Discov* 2019; **18**: 219-234 [PMID: 30679806 DOI: 10.1038/s41573-018-0011-2]
57 **Lewczuk N**, Zdebik A, Bogusławska J. Interferon Alpha 2a and 2b in Ophthalmology: A Review. *J Interferon Cytokine Res* 2019; **39**: 259-272 [PMID: 30950678 DOI: 10.1089/jir.2018.0125]
58 **Xue JH**, Zhu HH, Wang J, Chen Z. Interferon-associated retinopathy risk in patients with diabetes and hypertensive hepatitis C. *World J Gastroenterol* 2014; **20**: 7505-7513 [PMID: 24966622 DOI: 10.3748/wjg.v20.i23.7505]
59 **ISAACS A**, LINDENMANN J. Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci* 1957; **147**: 258-267 [PMID: 13465720 DOI: 10.1098/rspb.1957.0048]
60 **Goodbourn S**, Didcock L, Randall RE. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *J Gen Virol* 2000; **81**: 2341-2364 [PMID: 10993923 DOI: 10.1099/0022-1317-81-10-2341]
61 **Schoggins JW**. Interferon-stimulated genes: roles in viral pathogenesis. *Curr Opin Virol* 2014; **6**: 40-46 [PMID: 24713352 DOI: 10.1016/j.coviro.2014.03.006]
62 **Stifter SA**, Feng CG. Interfering with immunity: detrimental role of type I IFNs during infection. *J Immunol* 2015; **194**: 2455-2465 [PMID: 25747907 DOI: 10.4049/jimmunol.1402794]
63 **Pinto EF**, Andrade C. Interferon-Related Depression: A Primer on Mechanisms, Treatment, and Prevention of a Common Clinical Problem. *Curr Neuropharmacol* 2016; **14**: 743-748 [PMID: 26733280 DOI: 10.2174/1570159x14666160106155129]
64 **George PM**, Badiger R, Alazawi W, Foster GR, Mitchell JA. Pharmacology and therapeutic potential of interferons. *Pharmacol Ther* 2012; **135**: 44-53 [PMID: 22484806 DOI: 10.1016/j.pharmthera.2012.03.006]
65 **Anguille S**, Lion E, Willemen Y, Van Tendeloo VF, Berneman ZN, Smits EL. Interferon-α in acute myeloid leukemia: an old drug revisited. *Leukemia* 2011; **25**: 739-748 [PMID: 21274002 DOI: 10.1038/leu.2010.324]
66 **Kirkwood JM**, Strawderman MH, Ernstoff MS, Smith TJ, Borden EC, Blum RH. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J Clin Oncol* 1996; **14**: 7-17 [PMID: 8558223 DOI: 10.1200/JCO.1996.14.1.7]
67 **Jakimovski D**, Kolb C, Ramanathan M, Zivadinov R, Weinstock-Guttman B. Interferon β for Multiple Sclerosis. *Cold Spring Harb Perspect Med* 2018; **8** [PMID: 29311124 DOI: 10.1101/cshperspect.a032003]
68 **Pestka S**, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. *Immunol Rev* 2004; **202**: 8-32 [PMID: 15546383 DOI: 10.1111/j.0105-2896.2004.00204.x]
69 **Gysemans CA**, Ladrière L, Callewaert H, Rasschaert J, Flamez D, Levy DE, Matthys P, Eizirik DL,

Mathieu C. Disruption of the gamma-interferon signaling pathway at the level of signal transducer and activator of transcription-1 prevents immune destruction of beta-cells. *Diabetes* 2005; **54**: 2396-2403 [PMID: 16046307 DOI: 10.2337/diabetes.54.8.2396]

70 **Crosse KM**, Monson EA, Beard MR, Helbig KJ. Interferon-Stimulated Genes as Enhancers of Antiviral Innate Immune Signaling. *J Innate Immun* 2018; **10**: 85-93 [PMID: 29186718 DOI: 10.1159/000484258]

71 **Iwasaki A**. A virological view of innate immune recognition. *Annu Rev Microbiol* 2012; **66**: 177-196 [PMID: 22994491 DOI: 10.1146/annurev-micro-092611-150203]

72 **Schoggins JW**. Interferon-Stimulated Genes: What Do They All Do? *Annu Rev Virol* 2019; **6**: 567-584 [PMID: 31283436 DOI: 10.1146/annurev-virology-092818-015756]

73 **Schoggins JW**, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol* 2011; **1**: 519-525 [PMID: 22328912 DOI: 10.1016/j.coviro.2011.10.008]

74 **Marroqui L**, Dos Santos RS, Op de Beeck A, Coomans de Brachène A, Marselli L, Marchetti P, Eizirik DL. Interferon-α mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. *Diabetologia* 2017; **60**: 656-667 [PMID: 28062922 DOI: 10.1007/s00125-016-4201-3]

75 **Zhang S**, Kohli K, Black RG, Yao L, Spadinger SM, He Q, Pillarisetty VG, Cranmer LD, Van Tine BA, Yee C, Pierce RH, Riddell SR, Jones RL, Pollack SM. Systemic Interferon-γ Increases MHC Class I Expression and T-cell Infiltration in Cold Tumors: Results of a Phase 0 Clinical Trial. *Cancer Immunol Res* 2019; **7**: 1237-1243 [PMID: 31171504 DOI: 10.1158/2326-6066.CIR-18-0940]

76 **Mosser DM**. The many faces of macrophage activation. *J Leukoc Biol* 2003; **73**: 209-212 [PMID: 12554797 DOI: 10.1189/jlb.0602325]

77 **Carinelli S**, Xufré C, Martí M, Pividori MI. Interferon gamma transcript detection on T cells based on magnetic actuation and multiplex double-tagging electrochemical genosensing. *Biosens Bioelectron* 2018; **117**: 183-190 [PMID: 29902634 DOI: 10.1016/j.bios.2018.05.030]

78 **Castro F**, Cardoso AP, Gonçalves RM, Serre K, Oliveira MJ. Interferon-Gamma at the Crossroads of Tumor Immune Surveillance or Evasion. *Front Immunol* 2018; **9**: 847 [PMID: 29780381 DOI: 10.3389/fimmu.2018.00847]

79 **Alspach E**, Lussier DM, Schreiber RD. Interferon γ and Its Important Roles in Promoting and Inhibiting Spontaneous and Therapeutic Cancer Immunity. *Cold Spring Harb Perspect Biol* 2019; **11** [PMID: 29661791 DOI: 10.1101/cshperspect.a028480]

80 **Burke JD**, Young HA. IFN-γ: A cytokine at the right time, is in the right place. *Semin Immunol* 2019; **43**: 101280 [PMID: 31221552 DOI: 10.1016/j.smim.2019.05.002]

81 **Colli ML**, Hill JLE, Marroquí L, Chaffey J, Dos Santos RS, Leete P, Coomans de Brachène A, Paula FMM, Op de Beeck A, Castela A, Marselli L, Krogvold L, Dahl-Jorgensen K, Marchetti P, Morgan NG, Richardson SJ, Eizirik DL. PDL1 is expressed in the islets of people with type 1 diabetes and is up-regulated by interferons-α and-γ *via* IRF1 induction. *EBioMedicine* 2018; **36**: 367-375 [PMID: 30269996 DOI: 10.1016/j.ebiom.2018.09.040]

82 **Whitehead M**, Wickremasinghe S, Osborne A, Van Wijngaarden P, Martin KR. Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies. *Expert Opin Biol Ther* 2018; **18**: 1257-1270 [PMID: 30408422 DOI: 10.1080/14712598.2018.1545836]

83 **Sinclair SH**, Schwartz SS. Diabetic Retinopathy-An Underdiagnosed and Undertreated Inflammatory, Neuro-Vascular Complication of Diabetes. *Front Endocrinol (Lausanne)* 2019; **10**: 843 [PMID: 31920963 DOI: 10.3389/fendo.2019.00843]

84 **Moura FM**, Miranda MMM, Mendes DP, Henrique NM, Braga BB. Glycemic control and the production of cytokines in diabetic patients with chronic periodontal disease. *Rgo Revista Gacha De Odontologia* 2015; **63**: 432-438

85 **Abdel-Moneim A**, Semmler M, Abdel-Reheim ES, Zanaty MI, Addaleel W. Association of glycemic status and interferon-γ production with leukocytes and platelet indices alterations in type2 diabetes. *Diabetes Metab Syndr* 2019; **13**: 1963-1969 [PMID: 31235122 DOI: 10.1016/j.dsx.2019.04.046]

86 **Demirtas L**, Degirmenci H, Akbas EM, Ozcicek A, Timuroglu A, Gurel A, Ozcicek F. Association of hematological indicies with diabetes, impaired glucose regulation and microvascular complications of diabetes. *Int J Clin Exp Med* 2015; **8**: 11420-11427 [PMID: 26379958]

87 **Wang N**, Liang H, Zen K. Molecular mechanisms that influence the macrophage m1-m2 polarization balance. *Front Immunol* 2014; **5**: 614 [PMID: 25506346 DOI: 10.3389/fimmu.2014.00614]

88 **Hume DA**. The Many Alternative Faces of Macrophage Activation. *Front Immunol* 2015; **6**: 370 [PMID: 26257737 DOI: 10.3389/fimmu.2015.00370]

89 **Wolfs IM**, Donners MM, de Winther MP. Differentiation factors and cytokines in the atherosclerotic plaque micro-environment as a trigger for macrophage polarisation. *Thromb Haemost* 2011; **106**: 763-771 [PMID: 21947328 DOI: 10.1160/TH11-05-0320]

90 **Sica A**, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* 2012; **122**: 787-795 [PMID: 22378047 DOI: 10.1172/JCI59643]

91 **Voest EE**, Kenyon BM, O'Reilly MS, Truitt G, D'Amato RJ, Folkman J. Inhibition of angiogenesis in vivo by interleukin 12. *J Natl Cancer Inst* 1995; **87**: 581-586 [PMID: 7538593 DOI: 10.1093/jnci/87.8.581]

92 **Tugues S**, Burkhard SH, Ohs I, Vrohlings M, Nussbaum K, Vom Berg J, Kulig P, Becher B. New

insights into IL-12-mediated tumor suppression. *Cell Death Differ* 2015; **22**: 237-246 [PMID: 25190142 DOI: 10.1038/cdd.2014.134]

93 **Zhou Y**, Yoshida S, Kubo Y, Kobayashi Y, Nakama T, Yamaguchi M, Ishikawa K, Nakao S, Ikeda Y, Ishibashi T, Sonoda KH. Interleukin-12 inhibits pathological neovascularization in mouse model of oxygen-induced retinopathy. *Sci Rep* 2016; **6**: 28140 [PMID: 27312090 DOI: 10.1038/srep28140]

94 **Taylor S**, Mehina E, White E, Reeson P, Yongblah K, Doyle KP, Brown CE. Suppressing Interferon-γ Stimulates Microglial Responses and Repair of Microbleeds in the Diabetic Brain. *J Neurosci* 2018; **38**: 8707-8722 [PMID: 30201775 DOI: 10.1523/JNEUROSCI.0734-18.2018]

95 **Pan W**, Banks WA, Kastin AJ. Permeability of the blood-brain and blood-spinal cord barriers to interferons. *J Neuroimmunol* 1997; **76**: 105-111 [PMID: 9184639 DOI: 10.1016/s0165-5728(97)00034-9]

96 **Zhang Y**, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci* 2014; **34**: 11929-11947 [PMID: 25186741 DOI: 10.1523/JNEUROSCI.1860-14.2014]

97 **Du J**, Dong W, Li H, Li B, Liu X, Kong Q, Sun W, Sun T, Ma P, Cui Y, Kang P. Protective effects of IFN-γ on the kidney of type- 2 diabetic KKAy mice. *Pharmacol Rep* 2018; **70**: 607-613 [PMID: 29684848 DOI: 10.1016/j.pharep.2017.12.009]

98 **Du J**, Wang L, Liu L, Fan Q, Yao L, Cui Y, Kang P, Zhao H, Feng X, Gao H. IFN-γ suppresses the high glucose-induced increase in TGF-β1 and CTGF synthesis in mesangial cells. *Pharmacol Rep* 2011; **63**: 1137-1144 [PMID: 22180355 DOI: 10.1016/s1734-1140(11)70632-6]

99 **Lv W**, Booz GW, Wang Y, Fan F, Roman RJ. Inflammation and renal fibrosis: Recent developments on key signaling molecules as potential therapeutic targets. *Eur J Pharmacol* 2018; **820**: 65-76 [PMID: 29229532 DOI: 10.1016/j.ejphar.2017.12.016]

100 **Mitchell P**, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet* 2018; **392**: 1147-1159 [PMID: 30303083 DOI: 10.1016/S0140-6736(18)31550-2]

101 **Chistiakov DA**, Myasoedova VA, Revin VV, Orekhov AN, Bobryshev YV. The impact of interferon-regulatory factors to macrophage differentiation and polarization into M1 and M2. *Immunobiology* 2018; **223**: 101-111 [PMID: 29032836 DOI: 10.1016/j.imbio.2017.10.005]

102 **Brown J**, Wallet MA, Krastins B, Sarracino D, Goodenow MM. Proteome bioprofiles distinguish between M1 priming and activation states in human macrophages. *J Leukoc Biol* 2010; **87**: 655-662 [PMID: 20007246 DOI: 10.1189/jlb.0809570]

103 **Cao X**, Shen D, Patel MM, Tuo J, Johnson TM, Olsen TW, Chan CC. Macrophage polarization in the maculae of age-related macular degeneration: a pilot study. *Pathol Int* 2011; **61**: 528-535 [PMID: 21884302 DOI: 10.1111/j.1440-1827.2011.02695.x]

104 **Liu B**, Faia L, Hu M, Nussenblatt RB. Pro-angiogenic effect of IFNgamma is dependent on the PI3K/mTOR/translational pathway in human retinal pigmented epithelial cells. *Mol Vis* 2010; **16**: 184-193 [PMID: 20157617 DOI: 10.1186/1471-2415-10-3]

105 **Mühl H**, Pfeilschifter J. Anti-inflammatory properties of pro-inflammatory interferon-gamma. *Int Immunopharmacol* 2003; **3**: 1247-1255 [PMID: 12890422 DOI: 10.1016/S1567-5769(03)00131-0]

106 **Battle TE**, Lynch RA, Frank DA. Signal transducer and activator of transcription 1 activation in endothelial cells is a negative regulator of angiogenesis. *Cancer Res* 2006; **66**: 3649-3657 [PMID: 16585190 DOI: 10.1158/0008-5472.CAN-05-3612]

107 **Kawano Y**, Matsui N, Kamihigashi S, Narahara H, Miyakawa I. Effects of interferon-gamma on secretion of vascular endothelial growth factor by endometrial stromal cells. *Am J Reprod Immunol* 2000; **43**: 47-52 [PMID: 10698041 DOI: 10.1111/j.8755-8920.2000.430109.x]

108 **Kleinman ME**, Yamada K, Takeda A, Chandrasekaran V, Nozaki M, Baffi JZ, Albuquerque RJ, Yamasaki S, Itaya M, Pan Y, Appukuttan B, Gibbs D, Yang Z, Karikó K, Ambati BK, Wilgus TA, DiPietro LA, Sakurai E, Zhang K, Smith JR, Taylor EW, Ambati J. Sequence- and target-independent angiogenesis suppression by siRNA *via* TLR3. *Nature* 2008; **452**: 591-597 [PMID: 18368052 DOI: 10.1038/nature06765]

109 **Vaziri K**, Schwartz SG, Relhan N, Kishor KS, Flynn HW Jr. New Therapeutic Approaches in Diabetic Retinopathy. *Rev Diabet Stud* 2015; **12**: 196-210 [PMID: 26676668 DOI: 10.1900/RDS.2015.12.196]

110 **Stitt AW**, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res* 2016; **51**: 156-186 [PMID: 26297071 DOI: 10.1016/j.preteyeres.2015.08.001]

111 **Kiladjian JJ**, Giraudier S, Cassinat B. Interferon-alpha for the therapy of myeloproliferative neoplasms: targeting the malignant clone. *Leukemia* 2016; **30**: 776-781 [PMID: 26601783 DOI: 10.1038/leu.2015.326]

112 **Lombardi A**, Tsomos E, Hammerstad SS, Tomer Y. Interferon alpha: The key trigger of type 1 diabetes. *J Autoimmun* 2018; **94**: 7-15 [PMID: 30115527 DOI: 10.1016/j.jaut.2018.08.003]

113 **Mahmoud MH**, Badr G, Badr BM, Kassem AU, Mohamed MS. Elevated IFN-alpha/beta levels in a streptozotocin-induced type I diabetic mouse model promote oxidative stress and mediate depletion of spleen-homing CD8+ T cells by apoptosis through impaired CCL21/CCR7 axis and IL-7/CD127 signaling. *Cell Signal* 2015; **27**: 2110-2119 [PMID: 26192098 DOI: 10.1016/j.cellsig.2015.07.005]

114 **Marro BS**, Ware BC, Zak J, de la Torre JC, Rosen H, Oldstone MB. Progression of type 1 diabetes from the prediabetic stage is controlled by interferon-α signaling. *Proc Natl Acad Sci USA* 2017;

114: 3708-3713 [PMID: 28325871 DOI: 10.1073/pnas.1700878114]
115 **Wang YL**, Wang K, Yu SJ, Li Q, Li N, Lin PY, Li MM, Guo JY. Association of the TLR4 signaling pathway in the retina of streptozotocin-induced diabetic rats. *Graefes Arch Clin Exp Ophthalmol* 2015; **253**: 389-398 [PMID: 25359392 DOI: 10.1007/s00417-014-2832-y]
116 **Gerber SA**, Pober JS. IFN-alpha induces transcription of hypoxia-inducible factor-1alpha to inhibit proliferation of human endothelial cells. *J Immunol* 2008; **181**: 1052-1062 [PMID: 18606657 DOI: 10.4049/jimmunol.181.2.1052]
117 **Cheung CM**, Vania M, Ang M, Chee SP, Li J. Comparison of aqueous humor cytokine and chemokine levels in diabetic patients with and without retinopathy. *Mol Vis* 2012; **18**: 830-837 [PMID: 22511846 DOI: 10.1186/1471-2415-12-6]
118 **Chen H**, Zhang X, Liao N, Wen F. Assessment of biomarkers using multiplex assays in aqueous humor of patients with diabetic retinopathy. *BMC Ophthalmol* 2017; **17**: 176 [PMID: 28969616 DOI: 10.1186/s12886-017-0572-6]
119 **Mérida S**, Palacios E, Navea A, Bosch-Morell F. New Immunosuppressive Therapies in Uveitis Treatment. *Int J Mol Sci* 2015; **16**: 18778-18795 [PMID: 26270662 DOI: 10.3390/ijms160818778]
120 **Leibovitch I**, Loewenstein A, Alster Y, Rosenblatt I, Lazar M, Yassur Y, Rubinstein A. Interferon alpha-2a for proliferative diabetic retinopathy after complete laser panretinal photocoagulation treatment. *Ophthalmic Surg Lasers Imaging* 2004; **35**: 16-22 [PMID: 14750758 DOI: 10.1097/01.IOP.0000105516.42494.39]
121 **Skowsky WR**, Siddiqui T, Hodgetts D, Lambrou FH Jr, Stewart MW, Foster MT Jr. A pilot study of chronic recombinant interferon-alfa 2a for diabetic proliferative retinopathy: metabolic effects and opthalmologic effects. *J Diabetes Complications* 1996; **10**: 94-99 [PMID: 8777337 DOI: 10.1016/1056-8727(95)00005-4]
122 **Cellini M**, Balducci N, Strobbe E, Campos EC. Subtenon injection of natural leukocyte interferon α-2a in diabetic macular edema: a case report. *BMC Ophthalmol* 2013; **13**: 63 [PMID: 24165224 DOI: 10.1186/1471-2415-13-63]
123 **Panetta JD**, Gilani N. Interferon-induced retinopathy and its risk in patients with diabetes and hypertension undergoing treatment for chronic hepatitis C virus infection. *Aliment Pharmacol Ther* 2009; **30**: 597-602 [PMID: 19549263 DOI: 10.1111/j.1365-2036.2009.04071.x]
124 **Mousa N**, Besheer T, Gad Y, Elbendary A, Mokbel T, Abdel-Aziz A. Is combination therapy interferon and ribavirin in patients with chronic hepatitis C infection toxic for eyes? *J Ocul Pharmacol Ther* 2013; **29**: 345-348 [PMID: 23113644 DOI: 10.1089/jop.2012.0169]
125 **Ghany MG**, Morgan TR; AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology* 2020; **71**: 686-721 [PMID: 31816111 DOI: 10.1002/hep.31060]
126 **Vujosevic S**, Tempesta D, Noventa F, Midena E, Sebastiani G. Pegylated interferon-associated retinopathy is frequent in hepatitis C virus patients with hypertension and justifies ophthalmologic screening. *Hepatology* 2012; **56**: 455-463 [PMID: 22331668 DOI: 10.1002/hep.25654]
127 **Tu KL**, Bowyer J, Schofield K, Harding S. Severe interferon associated retinopathy. *Br J Ophthalmol* 2003; **87**: 247-248 [PMID: 12543766 DOI: 10.1136/bjo.87.2.247]
128 **Guyer DR**, Tiedeman J, Yannuzzi LA, Slakter JS, Parke D, Kelley J, Tang RA, Marmor M, Abrams G, Miller JW. Interferon-associated retinopathy. *Arch Ophthalmol* 1993; **111**: 350-356 [PMID: 8447745 DOI: 10.1001/archopht.1993.01090030068041]
129 **Konrad T**, Vicini P, Zeuzem S, Toffolo G, Briem D, Lormann J, Herrmann G, Wittmann D, Lenz T, Kusterer K, Teuber G, Cobelli C, Usadel KH. Interferon-alpha improves glucose tolerance in diabetic and non-diabetic patients with HCV-induced liver disease. *Exp Clin Endocrinol Diabetes* 1999; **107**: 343-349 [PMID: 10543410 DOI: 10.1055/s-0029-1212124]
130 **Butler NJ**, Suhler EB, Rosenbaum JT. Interferon alpha 2b in the treatment of uveitic cystoid macular edema. *Ocul Immunol Inflamm* 2012; **20**: 86-90 [PMID: 22409560 DOI: 10.3109/09273948.2011.645989]
131 **Sobaci G**, Erdem U, Durukan AH, Erdurman C, Bayer A, Köksal S, Karagul S, Bayraktar MZ. Safety and effectiveness of interferon alpha-2a in treatment of patients with Behçet's uveitis refractory to conventional treatments. *Ophthalmology* 2010; **117**: 1430-1435 [PMID: 20417563 DOI: 10.1016/j.ophtha.2009.11.022]
132 **Zanjani H**, Aminifard MN, Ghafourian A, Pourazizi M, Maleki A, Arish M, Shahrakipoor M, Rohani MR, Abrishami M, Khafri Zare E, Barzegar Jalali F. Comparative Evaluation of Tacrolimus Versus Interferon Alpha-2b Eye Drops in the Treatment of Vernal Keratoconjunctivitis: A Randomized, Double-Masked Study. *Cornea* 2017; **36**: 675-678 [PMID: 28399035 DOI: 10.1097/ICO.0000000000001200]
133 **Maleki A**, Stephenson AP, Hajizadeh F. Topical Interferon Alpha 2b in the Treatment of Refractory Diabetic Macular Edema. *J Ophthalmic Vis Res* 2020; **15**: 453-458 [PMID: 33133435 DOI: 10.18502/jovr.v15i4.7785]
134 **Kim SE**, Salvi SM. Immunoreduction of ocular surface tumours with intralesional interferon alpha-2a. *Eye (Lond)* 2018; **32**: 460-462 [PMID: 28937145 DOI: 10.1038/eye.2017.196]
135 **Tan JC**, Tat LT, Coroneo MT. Treatment of partial limbal stem cell deficiency with topical interferon α-2b and retinoic acid. *Br J Ophthalmol* 2016; **100**: 944-948 [PMID: 26508779 DOI: 10.1136/bjophthalmol-2015-307411]
136 **Karp CL**, Galor A, Chhabra S, Barnes SD, Alfonso EC. Subconjunctival/perilesional recombinant

interferon α2b for ocular surface squamous neoplasia: a 10-year review. *Ophthalmology* 2010; **117**: 2241-2246 [PMID: 20619462 DOI: 10.1016/j.ophtha.2010.03.052]

137 **Blasi MA**, Tiberti AC, Valente P, Laguardia M, Sammarco MG, Balestrazzi A, Larocca LM, Balestrazzi E. Intralesional interferon-α for conjunctival mucosa-associated lymphoid tissue lymphoma: long-term results. *Ophthalmology* 2012; **119**: 494-500 [PMID: 22226154 DOI: 10.1016/j.ophtha.2011.09.008]

138 **Erdem U**, Kerimoglu H, Gundogan FC, Dagli S. Treatment of Mooren's ulcer with topical administration of interferon alfa 2a. *Ophthalmology* 2007; **114**: 446-449 [PMID: 17198731 DOI: 10.1016/j.ophtha.2006.09.024]

139 **Minkovitz JB**, Pepose JS. Topical interferon alpha-2a treatment of herpes simplex keratitis resistant to multiple antiviral medications in an immunosuppressed patient. *Cornea* 1995; **14**: 326-330 [PMID: 7600820 DOI: 10.1097/00003226-199505000-00017]

140 **Sobaci G**, Bayraktar Z, Bayer A. Interferon alpha-2a treatment for serpiginous choroiditis. *Ocul Immunol Inflamm* 2005; **13**: 59-66 [PMID: 15804771 DOI: 10.1080/09273940490518865]

141 **Engler C**, Sander B, Villumsen J, Lund-Andersen H. Interferon alfa-2a modifies the course of subfoveal and juxtafoveal choroidal neovascularisation. *Br J Ophthalmol* 1994; **78**: 749-753 [PMID: 7528534 DOI: 10.1136/bjo.78.10.749]

142 **Brasnu E**, Wechsler B, Bron A, Charlotte F, Bliefeld P, Lehoang P, Marcelin AG, Bodaghi B. Efficacy of interferon-alpha for the treatment of Kaposi's sarcoma herpesvirus-associated uveitis. *Am J Ophthalmol* 2005; **140**: 746-748 [PMID: 16226536 DOI: 10.1016/j.ajo.2005.04.025]

143 **Fardeau C**, Simon A, Rodde B, Viscogliosi F, Labalette P, Looten V, Tézenas du Montcel S, LeHoang P. Interferon-alpha2a and Systemic Corticosteroid in Monotherapy in Chronic Uveitis: Results of the Randomized Controlled BIRDFERON Study. *Am J Ophthalmol* 2017; **177**: 182-194 [PMID: 28315319 DOI: 10.1016/j.ajo.2017.03.001]

144 **Couret C**, Servant M, Lebranchu P, Hamidou M, Weber M. Efficacy and Safety of Interferon Alpha 2A and Pegylated Interferon Alpha 2A in Inflammatory Macular Edema. *Ocul Immunol Inflamm* 2020; **28**: 329-336 [PMID: 31821064 DOI: 10.1080/09273948.2019.1604001]

145 **Poothullil AM**, Colby KA. Topical medical therapies for ocular surface tumors. *Semin Ophthalmol* 2006; **21**: 161-169 [PMID: 16912014 DOI: 10.1080/08820530500351694]

146 **Krilis M**, Tsang H, Coroneo M. Treatment of conjunctival and corneal epithelial neoplasia with retinoic acid and topical interferon alfa-2b: long-term follow-up. *Ophthalmology* 2012; **119**: 1969-1973 [PMID: 22704834 DOI: 10.1016/j.ophtha.2012.03.045]

147 **Bolek B**, Wylęgała A, Teper S, Kokot J, Wylęgała E. Treatment of conjunctival papilloma with topical interferon alpha-2b - case report. *Medicine (Baltimore)* 2020; **99**: e19181 [PMID: 32049852 DOI: 10.1097/MD.0000000000019181]

148 **Kivelä T**, Suciu S, Hansson J, Kruit WH, Vuoristo MS, Kloke O, Gore M, Hahka-Kemppinen M, Parvinen LM, Kumpulainen E, Humblet Y, Pyrhönen S. Bleomycin, vincristine, lomustine and dacarbazine (BOLD) in combination with recombinant interferon alpha-2b for metastatic uveal melanoma. *Eur J Cancer* 2003; **39**: 1115-1120 [PMID: 12736111 DOI: 10.1016/s0959-8049(03)00132-1]

149 **Celiker H**, Kazokoglu H, Direskeneli H. Long-Term Efficacy of Pegylated Interferon Alpha-2b in Behçet's Uveitis: A Small Case Series. *Ocul Immunol Inflamm* 2019; **27**: 15-22 [PMID: 28700247 DOI: 10.1080/09273948.2017.1332768]

150 **Qian Z**, Fardeau C, Cardoso JN, Jellab B, Fan X, LeHoang P. Effect of interferon α2a in cystoid macular edema due to intraocular infection. *Eur J Ophthalmol* 2015; **25**: 431-436 [PMID: 25790810 DOI: 10.5301/ejo.5000591]

151 **Sundmacher R**, Mattes A, Neumann-Haefelin D, Adolf G, Kruss B. The potency of interferon-alpha 2 and interferon-gamma in a combination therapy of dendritic keratitis. A controlled clinical study. *Curr Eye Res* 1987; **6**: 273-276 [PMID: 3030650 DOI: 10.3109/02713688709020104]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 954-974

DOI: 10.4239/wjd.v12.i7.954

ISSN 1948-9358 (online)

REVIEW

Ejaculatory dysfunction in men with diabetes mellitus

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Author contributions: Mostafa T and Abdel-Hamid IA contributed equally to this work in all its aspects.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Manuscript source: Invited manuscript

Specialty type: Andrology

Country/Territory of origin: Egypt

Peer-review report's scientific quality classification

Grade A (Excellent): 0

Grade B (Very good): B

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Abstract

Diabetes mellitus (DM) is a metabolic disorder that is characterized by elevated blood glucose levels due to absolute or relative insulin deficiency, in the background of β-cell dysfunction, insulin resistance, or both. Such chronic hyperglycemia is linked to long-term damage to blood vessels, nerves, and various organs. Currently, the worldwide burden of DM and its complications is in increase. Male sexual dysfunction is one of the famous complications of DM, including abnormal orgasmic/ejaculatory functions, desire/libido, and erection. Ejaculatory dysfunction encompasses several disorders related to DM and its complications, such as premature ejaculation, anejaculation (AE), delayed ejaculation, retrograde ejaculation (RE), ejaculatory pain, anesthetic ejaculation, decreased ejaculate volume, and decreased force of ejaculation. The problems linked to ejaculatory dysfunction may extend beyond the poor quality of life in diabetics as both AE and RE are alleged to alter the fertility potential of these patients. However, although both diabetes patients and their physicians are increasingly aware of diabetic ejaculatory dysfunction, this awareness still lags behind that of other diabetes complications. Therefore, all these disorders should be looked for thoroughly during the clinical evaluation of diabetic men. Besides, introducing the suitable option and/or maneuvers to treat these disorders should be tailored according to each case. This review aimed to explore the most important findings regarding ejaculatory dysfunction in diabetes from pre-clinical and clinical perspectives.

Key Words: Diabetes mellitus; Ejaculation; Anejaculation; Retrograde ejaculation; Semen

Core Tip: Male sexual dysfunction is a famous complication of diabetes mellitus (DM),

Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 30, 2021
Peer-review started: January 30, 2021
First decision: May 3, 2021
Revised: May 6, 2021
Accepted: June 15, 2021
Article in press: June 15, 2021
Published online: July 15, 2021

P-Reviewer: Kamalanathan S
S-Editor: Zhang H
L-Editor: Filipodia
P-Editor: Wang LL

including abnormal orgasmic/ejaculatory function, desire/libido, and erection. DM-related ejaculatory dysfunction encompasses several disorders such as premature ejaculation, anejaculation (AE), delayed ejaculation, retrograde ejaculation (RE), ejaculatory pain, anesthetic ejaculation, decreased ejaculate volume, and decreased force of ejaculation. The problems linked to ejaculatory dysfunction may lead to poor quality of life as both AE and RE are alleged to alter their fertility potential. All these disorders should be looked for thoroughly during the clinical evaluation of diabetic men.

Citation: Mostafa T, Abdel-Hamid IA. Ejaculatory dysfunction in men with diabetes mellitus. *World J Diabetes* 2021; 12(7): 954-974
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/954.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.954

INTRODUCTION

Ejaculation is a complex, coordinated sequence of mechanisms and reflexes compared to erection. It requires coordinated innervation of the sympathetic and parasympathetic systems in addition to somatic innervation. In essence, it is intermediated by a multifaceted neural control system including sensory receptors, afferent pathways, cerebral sensory areas, cerebral motor centers, spinal motor centers, and efferent pathways. Likewise, this process comprises complex neurochemical interplay between central serotonergic and dopaminergic neurons, with involvement of cholinergic, adrenergic, oxytocinergic, and gamma-aminobutyric acidergic neurons[1]. However, ejaculation entails two diverse sequential coordinated phases, seminal emission and ejaculation proper, each of them involving diverse anatomic structures[2].

The sympathetic nervous system is accountable for bladder neck closure and emission, whereas the somatic nervous system is accountable for the contraction of the pelvic muscles and the bulbourethral and ischiocavernosal muscles[3]. Additionally, the external urinary bladder sphincter as well as the perineal periurethral muscles are under somatic control[4]. Emission process is characterized by closure of the bladder neck and contraction of smooth muscles across the seminal tract aiming of propulsion of the ejaculate constituents into the posterior urethra where they are mixed with spermatozoa to form seminal fluid[5]. This phase is activated by augmented sexual arousal and the peripheral sex-related stimuli. The sympathetic centers, found within the intermediolateral and the intermediomedial cell columns in lamina VII of segments T_{12}-L_2, supply the smooth muscles of the male accessory glands, seminal tract, and urinary bladder neck[6]. Before emission, there are secretions from the distal epididymis, seminal vesicles (SVs), and the prostate supporting emission. Neural control of those secretions is mediated by the cholinergic post-ganglionic, sympathetic, and parasympathetic fibers, derived from the pelvic plexus[6-8]. The emission phase is under the cerebral control and can be affected by the physical or visual erotic stimulation[9].

The ejaculation proper phase follows emission and refers to the relaxation of the external urinary sphincter followed by repetitive contractions of the bulbospongiosus, ischiocavernosus, and the pelvic striated muscles leading to expulsion of seminal fluid out of the urethra[10]. Motor neurons controlling the pelvic and perineal striated muscles are situated in the ventral horn of segments S_2-S_4 in Onuf's nucleus[11]. Both emission and ejaculation proper occur in coherence to bring normal antegrade ejaculation. Although the precise trigger for the ejection phase is not yet known, it is believed that the filling of the posterior urethra urges the urethral-muscular reflex boosted by sensory inputs-somatosensory, visceral sensory, or proprioceptive-to the spinal control center might trigger the onset of ejection. Harmonization between autonomic and somatic neurons for emission as well as ejection is supposed to be controlled by a group of lumbar spinothalamic interneurons called "spinal ejaculation generator (SEG)"[12]. In rats, these interneurons are situated in lamina X and the medial part of lamina VII of the gray matter in the L_3-L_4[13]. In humans, neurohistologic data, in addition to the clinical observations, have established the existence of SEG in L_3-L_5 segments[14].

Exact knowledge on hormonal control of ejaculation is still lacking. Studies have shown an oxytocin surge during male sexual activity, peaking during or soon after ejaculation[15]. Additionally, oxytocin's contractile effect concerning the seminal tract in humans appears to be weaker than in the animal models[16]. Moreover, estrogens have been demonstrated to take part in the peripheral regulation of epididymal contractility[17]. Furthermore, prolactin was also noted to increase around orgasm with oxytocin suggesting that it may serve as a neuroendocrine reproductive reflex for peripheral reproductive organs[18,19]. Androgens are deeply elaborated in the ejaculation process[20]. The effects of androgens are not limited to the fact that the development of the epididymis, vas deferens, and SVs is dependent on androgens, but also, spinal nuclei elaborated in the control of ejaculation, such as the nucleus of the bulbocavernosus nerve, are androgen-dependent[21], as are the muscles of the pelvic floor[22,23]. However, the dynamic and complex interplay among androgens, growth factors, and genes in the ejaculatory process is less well understood.

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia due to defects in insulin secretion and/or action[24]. The worldwide burden of DM and its complications are presently in an increase. DM is usually associated with long-term dysfunction of blood vessels, nerves, and several organs including the epididymis, vas deferens (VD), SV, prostate, and urethra[25-29]. Although both diabetes patients and their physicians are increasingly aware of diabetic ejaculatory dysfunction, this awareness still lags behind that of other diabetes complications. The problems linked to the ejaculatory dysfunction may extend beyond the poor quality of life in diabetics. Because anejaculation (AE) and retrograde ejaculation (RE) are believed to alter the fertility potential of patients[25,30]. Thus, the careful diagnosis and timely management of these cases together are of extreme importance. Therefore, this narrative review aims to explore the most important findings regarding ejaculatory dysfunction in diabetes from pre-clinical and clinical perspectives.

METHODS

We searched the PUBMED/MEDLINE, Scopus, Academic Search Complete database, Google Scholar, Cochrane Library, EMBASE, ProQuest, and CINAHL databases from inception to December 2020, for relevant studies. The literature search included these terms (with synonyms and closely related words): type 1 and type 2 diabetes mellitus; or hyperglycemia; or insulin resistance; and ejaculatory dysfunction; or premature ejaculation (PE); or delayed ejaculation (DE); or AE; or RE; or ejaculatory pain (EP); or anesthetic ejaculation; or spontaneous ejaculation (SE); or sexual dysfunction; or male sexual function; or epididymis; or VD; or SVs; or prostate; bladder neck; or urethra. The search was not limited by study design but restricted to English-language periodicals. Further studies were recognized by examining the reference lists of all retrieved articles.

ANIMAL STUDIES

In an attempt to understand the influence of DM on ejaculatory function, we have identified 18 studies (Table 1) reporting on the association between experimental DM and ejaculatory dysfunction. Generally, it seems that ejaculatory dysfunction after experimental DM is inexplicable because it ranges from PE, normal ejaculatory performance, to AE. Although the duration of the diabetic state seems to be the major determinant of the development of ejaculatory dysfunction, other factors such as dissimilar animal species and strains, with a diversity of metabolic pathways, different models for inducing DM, small experimental groups, nuances in laboratory technique that may affect the results, and selection of outcome measures of the ejaculatory function. Nonetheless, the following different observations were noted: (1) 3 studies showed no sexual performance difference between diabetic and control rats[31-33]; (2) prolonged ejaculation latency (EL) was reported in 9 studies suggesting DE[34-42]. Notably, in 1 study diabetic rats showed a significantly decreased amount of secretions stored in the seminal vesicle indicating that streptozotocin (STZ)-induced diabetic might affect the cholinergic nerve endings that are situated at the rich glandular epithelium[38]; (3) 0% ejaculations (rats did not achieve ejaculation) was noted in 4 reports suggesting AE[43-46]; and (4) reduced EL was reported in 2 reports suggesting PE[47,48]. These findings suggest not only that ejaculatory dysfunction in diabetic

Table 1 Ejaculatory behavior in diabetic animals

Ref.	Animal model	Effects observed on ejaculatory function	Type of treatment and response
Sach *et al*[31], 1982	STZ-induced diabetic rats	No sexual performance difference between diabetic and control rats.	
Clark[32], 1995	STZ-induced diabetic rats	No sexual performance difference between diabetic and control rats.	
Scarano *et al*[33], 2006	STZ-induced diabetic rats	No sexual performance differences in EL after 15 d.	
Steger *et al*[34], 1990	STZ-induced diabetic rats	Prolonged EL (DE or AE)	Delayed insulin replacement (4 wk) cannot prevent ejaculatory dysfunction.
Murray *et al*[35], 1992	Diabetic BB/WOR rats	Prolonged EL (DE or AE) after 28 wk	
McVary *et al*[36], 1997	Diabetic BB/WOR rats	Prolonged EL (DE or AE) after 40 wk, reduced number of ejaculations. No differences regarding serum testosterone, FSH, and LH.	
Ebiko *et al*[37], 2006	STZ-induced diabetic rats	Deteriorated spontaneous seminal emission after 5 wk. In 15 and 30 wk, occurrence of SSE was almost completely suppressed.	Early insulin replacement can prevent ejaculatory dysfunction.
Yonezawa *et al*[38], 2009	Streptozotocin (STZ)-induced diabetic rats	Deteriorated spontaneous seminal emission after 5 wk. Decreased ejaculated semen and decreased seminal vesicle fluid.	Early insulin replacement can prevent ejaculatory dysfunction. Once dysfunction occurs, insulin cannot restore it.
Suresh *et al*[39], 2012	STZ-induced diabetic rats	Prolonged EL suggesting DE. -Low serum testosterone.	Mucuna pruriens showed recovery of EL.
De *et al*[40], 2016	STZ-induced diabetic rats	Prolonged EL suggesting DE. -Low serum testosterone.	l-Norvaline (arginase inhibitor) reduced EL.
Shi *et al*[41], 2017	STZ-induced diabetic rats	Prolonged EL suggesting DE.	Lycium barbarum polysaccharide reduced EL.
Li *et al*[42], 2019	STZ-induced diabetic rats	Prolonged EL at 62 d suggesting DE.	No effect of vitexin (herb) on EL.
Lert-Amornpat *et al* [43], 2016	STZ-induced diabetic rats	Lack of copulatory behavior suggesting AE.	*Kaempferia parviflora* (herb) showed recovery of EL.
Fernández-Collazo *et al*[44], 1970	Rats with subtotal pancreatectomy	They did not AE.	
Hassan *et al*[45], 1993	STZ-induced diabetic rats	Rats exhibited AE in diabetics. -Low serum testosterone.	Sabeluzole treatment was beneficial to correct dysfunction.
Pontes *et al*[46], 2011	STZ-induced diabetic rats	Lack of the sperms ejaculated into the uterus. -Low serum testosterone.	Testosterone supplement did not restore ejaculatory function.
Ghaheri *et al*[47], 2018	STZ-induced diabetic rats	Shorten EL after 28 d suggesting PE.	Stevia Bertoni extract improved EL.
Minaz *et al*[48], 2019	STZ-induced diabetic rats	Shorten EL after 8 wk suggesting PE. -Low serum testosterone.	Inhibition of soluble epoxide hydrolase prolonged EL.

AE: Anejaculation; DE: Delayed ejaculation; EL: Ejaculation latency; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; PE: Premature ejaculation; STZ: Streptozotocin.

animals is variable but also may indicate that the dysfunction may occur during the early stage following experimental diabetes and becomes poorer as it progresses[34,38]. Additionally, early insulin replacement had been demonstrated to prevent ejaculatory dysfunction suggesting that insulin may play a role in controlling of seminal emission[34,37,38]. However, once the dysfunction occurs; delayed insulin replacement cannot restore normal ejaculatory function suggesting that long-term exposure to the hyperglycemia may lead to an irreversible ejaculatory dysfunction[34,38]. In this respect, it appears that insulin replacement may only delay rather than prevent changes in copulatory behavior[34].

The development of ejaculatory dysfunctions in animals is not limited to STZ-induced diabetic rats[34,37-40,43,45-48] but also have been demonstrated in STZ-induced diabetic mice[42,43], subtotal pancreatectomy-induced diabetic rats[44], and Bio Breeding Wistar Strain (BB/WOR) rats with spontaneous DM[35,36]. The major attributes of this latter strain (BB/WOR) are not only limited to the fact that its pathophysiology closely look like the development as well as the clinical features of type 1 diabetes in humans but also it lacks the artefactual end-organ changes seen with STZ such as angiopathy[36]. In other words, diabetic neuropathy is a prevailing feature of this model[49]. EL, the time of the first intromission until ejaculation, was the most frequently employed outcome measure to assess the ejaculatory function in these animal studies[34-36,39-43,47,48]. Other tools include spontaneous seminal emission test[37,38], measurement of seminal vesicle fluid[38], failure to recover spermatozoa in the female uterus[46], and numbers of ejaculations[36,43-45].

In the context of ejaculatory dysfunction associated with experimental DM, it would be of interest to review the potential effects of different therapeutic compounds to restore the ejaculatory function in STZ-induced diabetic mice. Nine studies were identified[39-43,45-48]. Animal studies have demonstrated that Mucuna pruriens[39], l-Norvaline (arginase inhibitor)[40], and Lycium barbarum polysaccharide[41] showed a reduction in the EL in those showing prolonged EL during the diabetic state. Additionally, both *Kaempferia parviflora*[43], and sabeluzole (benzothiazole derivative) [45], were found to be beneficial in correcting an ejaculatory condition. Moreover, Stevia Bertoni extracts and the soluble epoxide hydrolase inhibitor "trans-4-{4-[3-(4-trifluoromethoxyphenyl)-ureido] cyclohexyloxy} benzoic acid/t-TUCB"[47,48] showed prolongation of EL in those animals showing a reduction in EL. Unfortunately, both vitexin (bioactive flavonoids) and testosterone did not restore ejaculatory function in STZ-induced diabetic and mice, respectively. Finally, further experiments are needed to delineate better the effects of experimental diabetes on the ejaculatory function using a unified generally accepted easily measurable outcome measure.

ETIOPATHOPHOGENESIS OF DIABETIC EJACULATORY DYSFUNCTION

Animal studies assessed the effects of experimental diabetes on the end-organs of a seminal emission (epididymis, VD, SV, prostate, bladder neck, and urethra) have revealed that hyperglycemia is capable of altering the contractility of these organs either by modulating neurotransmitters release or by modifying the basal tone of the smooth muscle layers[50-52]. Interference with the normal function of these organs may therefore include central or peripheral mechanisms. Besides, these diabetes-associated changes may have general pathophysiological interest since ejaculatory dysfunction such as PE, DE, AE; or RE has been proven to be one of the complications of DM[25,27,28]. Moreover, animal models can deliver an important method to assess neural circuitry and molecular and cellular pathways in an organized setting. The following summarizes the major observations regarding the effects of experimental diabetes on the end-organs of seminal emission to understand the pathogenesis of diabetes-associated ejaculatory dysfunction: (1) Chronically STZ-diabetic rats and mice showed degenerative changes in the sympathetic supply of the VD leading to a decreased reaction to stimulation of their noradrenergic nerves and a supersensitivity to exogenous noradrenaline[50-54]. These findings suggest that a significant proportion of animals may have developed sympathetic neuropathy that may explain the prolongation of EL, reduction of the numbers of ejaculations, and the occurrence of AE; (2) The paravertebral thoracic ganglion cells of spontaneously diabetic BB rats exhibited a decreased number of synapses and the postganglionic fibers demonstrated increased glycogenosomes, axonal sequestration, and reduced axonal size suggesting an axonopathy in sympathetic nerves[49,53]. Additionally, this model showed a peripheral neuropathic change in both the hypogastric and motor pudendal nerve fibers suggesting that diabetic neuropathy is not only disturbed the emission phase but also may disrupt the ejection phase of the ejaculatory process[36]; (3) Reactive oxygen species (ROS) may be accountable for impaired sympathetic neurotransmission and the abnormal function of diabetic vas deferens in STZ-diabetic rats[54,55]; (4) There is evidence to suggest the presence of Ca channel hyperactivity in the smooth muscle of VD of STZ-induced diabetic rats possibly due to increased phosphatidylinositol turnover mediated by alpha 1-adrenoceptors[56,57]. These findings may reduce EL and explain the occurrence of PE; (5) Noradrenaline is the principal excitatory neurotransmitter in the internal urethral sphincter and augments closure of the bladder neck during ejaculation[58,59]. By principle, the combination of long-term

diabetic sympathetic neuropathy in animals[37,38,49] and external urethral sphincter relaxation dysfunction[60] may result in RE; (6) It has been demonstrated that long-term diabetes is associated with changes in serotonergic transmission in the rat brain including changes in several types of 5-HT receptors[61,62]. Theoretically, 3, 5-HT receptor subtypes (5-HT1A, 5-HT1B, and 5-HT2C) were assumed to mediate 5-HT's modulating activity on the ejaculation process. PE is associated with decreased neurotransmission of serotonin, 5-HT_{2C} receptor hyposensitivity, and 5-HT_{1A} receptor hypersensitivity[63]. Accordingly, ejaculatory dysfunction due to changes in serotonergic transmission among diabetic animals could be anticipated; (7) There is adequate evidence to suggest that experimental diabetes in different animal models (STZ rats, spontaneously diabetic BB rats, BB/WOR diabetic rats, and spontaneously diabetic Torii rats) is associated with low testosterone levels[64-70]. The pathogenesis of hypogonadism in diabetic animal models may include impaired hypothalamic or pituitary signaling[34,71,72], deficiency of gonadotropic hormones, or blockade of their actions[73,74], and/or primary Leydig cell defect in steroidogenesis due to lack of stimulating effect of insulin[74-76]. In the light of the foregoing, it might be assumed that there is a link between low testosterone levels and ejaculatory dysfunction. However, it has been shown that testosterone supplement is not able to bring back ejaculatory function in induced diabetic rats[46,77] suggesting that the deficiency of testosterone was not related directly to the diabetes-induced ejaculatory dysfunction in this experimental model. Although expression of androgen receptors are demonstrated at different levels of the ejaculatory process such as the medial preoptic area of the hypothalamus[78], smooth muscles of the male genital tract[79], and in the spinal nucleus of the bulbocavernosus muscle[80], it is thought that testosterone plays a much superior role in libido than the ejaculatory process and the physiological capacity for ejaculation is less sensitive to testosterone reduction than that for the desire[81,82]. In support of this notion, it has been shown that testosterone levels as low as 0.2 ng/mL, can support ejaculatory behavior in rats[83]; (8) It is possible that diabetic ejaculatory dysfunction might be a reflection of decreased sexual desire[36,48]. Although the classic description of diabetic erectile dysfunction showed preserved desire[84], it has been shown that sexual desire (mount frequency) is notably decreased in diabetic rats[34,45,85-87], but this possibility was unlikely because of the absence of improved ejaculation behavior among diabetic rats after testosterone therapy despite the improvement of their libido (mount behavior)[77]; and (9) Lastly other factors that may participate in the pathogenesis of ejaculatory dysfunction in experimental diabetes may include decreased body and reproductive organs weight [45,77]. However, the relationship between these variables and diabetic ejaculatory dysfunction remains incompletely understood.

Ejaculatory dysfunctions are established complications found with variable prevalence in men with diabetes. There is also a substantial contribution of human studies to the pathogenesis of diabetic ejaculatory dysfunction**.** The factors that have been postulated to influence the development of ejaculatory dysfunctions in DM are summarized (Tables 2 and 3). However, there are limited data regarding the weight of each mechanism in participating in the pathogenesis of different ejaculatory dysfunctions in diabetes patients.

DIABETES-RELATED EJACULATORY DYSFUNCTIONS

Ejaculatory dysfunctions encompass several disorders related to DM and its complications, such as PE, DE, AE, RE, ejaculatory pain, anesthetic ejaculation, decreased ejaculate volume, and decreased force of ejaculation. Up to 40% of men with diabetes may complain of ejaculatory dysfunction[99]. Of these PE, DE, AE, and RE are the most common and the focus of this discussion[100-113].

PE

There is contradictory evidence concerning the prevalence of PE in diabetics, with one study reporting increased PE prevalence in diabetic men compared to healthy controls (78.8% *vs* 47.5%, $P = 0.001$)[92]. On the other hand, a study by the Italian Society of Andrology pointed out to a protecting effect of diabetes on PE (6.2% *vs* 8.4%, adjusted odds ratio = 0.6, $P = 0.001$)[114]. This low prevalence was endorsed to delayed emission caused by diabetic sympathetic neuropathy of nerve fibers innervating the organs of emission (VD, SV, prostate). However, with the well-known relation between erectile dysfunction (ED) and PE that recognizes ED as the significant comorbidity of PE[115] and a higher prevalence of ED among diabetics, we could

Table 2 Possible pathophysiological mechanisms underlying ejaculatory dysfunctions in diabetes mellitus (animal studies)

Ref.	Postulated mechanisms	Possible ejaculatory dysfunction
McVary *et al*[36], Yagihashi *et al*[49]	Pathologic changes in the nerve supply of seminal tract due to accumulation of AGE increased ROS (sympathetic neuropathy)	Prolongation of EL
Tomlinson *et al*[50], Longhurst *et al*[51]	Decreased number of synapses in thoracic ganglia	Reduction of the numbers of ejaculations
Kaschube *et al*[52], Kamata *et al*[53]	Axonopathy in postganglionic sympathetic fibers	Disturbed the emission phase
Güneş *et al*[54], Tsounapi *et al*[55]	Neuropathic changes in hypogastric nerve and motor pudendal nerve fibers	Disruption of the ejection phase
Tomlinson *et al*[50], Longhurst *et al*[51], Kaschube *et al*[52], Kamata *et al*[53], Güneş *et al*[54]	Hypersensitivity (supersensitivity) of seminal tract smooth muscles to exogenous noradrenaline	Reduction of EL
Sakai *et al*[56], Sakai *et al*[57]	Hyperactivity of Ca channels in smooth muscles	Reduction of EL
Ebiko *et al*[37], Yonezawa *et al*[38]Yagihashi *et al*[49], Torimoto *et al*[60]	Sympathetic neuropathy and external urethral sphincter relaxation dysfunction	Disruption of bladder neck closure Disruption of AE
Sandrini *et al*[61], Abraham *et al*[62]	Changes in serotonergic transmission	Reduction or prolongation of EL
Seethalakshmi *et al*[71], Wolfe *et al*[72]	Impaired hypothalamic or pituitary signaling	Decreased sexual performance
Oksanen *et al*[73], Sudha *et al*[74]	Deficiency of gonadotropic hormones or blockade of their actions	Decreased sexual performance
Ballester *et al*[75], Neirijnck *et al*[76]	Decrease in number and function of Leydig cells	Decreased sexual performance
Neirijnck *et al*[76]	Defective testicular steroidogenesis	Decreased sexual performance
Kühn-Velten *et al*[64], Anderson *et al*[65], Ricci *et al*[66], Murray *et al*[67], Cameron *et al*[68], Ohta *et al*[69], Nakane *et al*[70]	Reduced serum levels of LH and testosterone (T)	Decreased sexual performance
McVary *et al*[36], Minaz *et al*[48], Steger *et al*[85], Al-Roujayee *et al*[86], Kashif *et al*[87]	Reduced libido	Decreased sexual performance, decreased mount frequency, and reduced EL
Hassan *et al*[45], Steger *et al*[77]	Reduced reproductive organ weight	Exact Effects on ejaculation still unknown

AGE: Advanced glycation end products; Ca: Calcium; DE: Delayed ejaculation; EL: Ejaculation latency; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; PE: Premature ejaculation; ROS: Reactive oxygen species; STZ: Streptozotocin; T: Testosterone.

speculate that diabetes might be considered a risk factor for PE. Anyway, most of the studies showed comparable prevalence between diabetic and non-diabetic persons[90,91,95,96]. Theoretically, diabetes-related PE is recognized to have a multi-factorial etiology (Tables 2 and 3). The lack of a significant difference in the prevalence of PE among men with/without diabetes might be attributed to socio-demographic factors, a multiplicity of different tools for measurement of PE such as self-reported PE, PE diagnostic tool (PEDT) score, self-reported, and stopwatch-recorded intravaginal ejaculation latency time (IELT) or to the presumed balance between the protective and detrimental factors inside the diabetes patients. Therefore, the true prevalence of PE in DM has not been firmly established. However, the PE subjects diagnosed with PEDT score or stopwatch IELT showed significant prevalence of diabetes[88,116] suggesting that DM is an important etiologic factor in acquired PE. Studies assessing whether PE in DM can be related to glycometabolic control and associated morbidities have also produced mixed results. While El-Sakka[91] and Malavige *et al*[93] have demonstrated that elongated duration of diabetes, poor metabolic control, and presence of cardiovascular disease are linked to increased risk of PE in type 2 DM, Other studies have found no such evidence in type 2 DM[92,97]. Additionally, in the subgroup of type 1 DM patients assessed for glucose variability, the PEDT score was associated with low blood glucose indices (r = 0.43; P = 0.01), but not with a standard deviation of blood glucose (r = 0.1, P = 0.6), mean amplitude of glycemic excursions (r = -0.1; P = 0.4), or high blood glucose indices (r = 0.1; P = 0.6) suggesting a link between glycemic excursions and PE[96]. This association between hypoglycemia and PE might be linked to increased adrenergic activity[117] or reduced serotoninergic activity[118]. Moreover, PE is known to be associated with diabetes-related ED as shown in different studies[91-93,97]. Likewise, one study suggests that 95% of Type 2 diabetes patients

Table 3 Possible pathophysiological mechanisms underlying ejaculatory dysfunctions in diabetes mellitus (human studies)

Ref.	Possible cause	Outcome
Premature ejaculation		
Culha *et al*[88]	Anxiety	Among PE patients with DM, 15% had anxiety
Culha *et al*[88], Khan *et al*[89]	Depression	Among PE patients with DM, 16.9% had depression
		Depression score Significantly higher among diabetic-related PE patients
Khan *et al*[89], Malavige *et al*[90]	Genetic and racial factors	Long tri-nucleotide repeats of the androgen receptor are related to the lowest IELT (PE)
		Asian men reported higher diabetic PE than European counterparts
El-Sakka[91]	Diabetic condition, duration of MD	> 10 yr of diabetes were 2.7 times as likely to report diabetic-related PE
	Poor glycemic control	Poor glycemic control were 9.6 times as likely to report PE
El-Sakka[91], Majzoub *et al*[92]	Having diabetic-related -erectile dysfunction (ED) and Cardiovascular diseases	Significant association between PE and cardiovascular diseases
Malavige *et al*[93], Malavige *et al*[90]		ED showed a significantly higher incidence of PE
Olamoyegun *et al*[94]		ED was strongly associated with PE odds ratio = 4.4
El-Sakka[91]	Having diabetic –related neuropathy	It is not associated with PE
Khan *et al*[89]	Total serum testosterone	Significantly higher among type 2 diabetic-related PE patients
Owiredu *et al*[95]		It correlates negatively with short IELT among type 2 DM
Bellastella *et al*[96]		No significant difference in type 1 diabetes
Delayed ejaculation and anejaculation		
Corona *et al*[97]	Depression	Severe depressive symptoms are associated with ejaculatory problems in DM
Ellenberg *et al*[25] , La Vignera *et al*[27] , Dinulovic *et al*[98]	Progressive autonomic neuropathy of the sympathetic nerves	Denervation leads to weak or loss of VD and SV peristaltic movements
Dunsmuir *et al*[99], Condorelli *et al*[100]		Abnormal inflammatory responses lead to alteration of the VD and SV peristaltic movements
La Vignera *et al*[101] , Pop-Busui *et al*[102]		Delayed /poor emission
		Absent emission
Haddad *et al*[26], Culver *et al*[103]	Calcification of vas deferens and seminal vesicles	Loss of their ability to contract as the smooth muscle is replaced by fibrotic, calcified tissue
Tsuno *et al*[104]		Delayed/poor emission
Hylmarova *et al*[29] , Corona *et al*[81]	Hypogonadism	No association between serum testosterone levels and ejaculation time in men self-reporting DE including diabetic patients
Paduch *et al*[82], Gianatti *et al*[105]		
Morgentaler *et al*[106]		T replacement is not associated with improvement in DE or AE
Burke *et al*[107], Corona *et al*[108]	Low sexual desire	DM is significantly associated with low sexual desire
Corona *et al*[109]		DE and AE are associated with low sexual desire
Retrograde ejaculation		
Klebanow *et al*[110]	Diabetic neuropathy (T10-L3)	Intact vasal and seminal vesicle contraction but incomplete simultaneous bladder neck closure leads to partial RE
Greene *et al*[111]		Intact vasal and seminal vesicle contraction and simultaneous complete lack of bladder neck closure leads to complete RE

Koyanagi[112] Cao *et al*[113]	External urethral sphincter relaxation dysfunction (triple parasympathetic-sympathetic-somatic innervation)	Lack of active external urethral sphincter relaxation leads to disruption of antegrade ejaculation

AE: Anejaculation; DE: Delayed ejaculation; DM: Diabetes Mellitus; EL: Ejaculation latency; ED: Erectile dysfunction; PE: Premature ejaculation; SV: Seminal vesicle; VD: Vas deferens.

with PE also reported ED[91] indicating that ED is the principal risk factor of PE in type 2 DM. A vicious cycle probably exists between ED and PE with each condition being deteriorated by the other[93]. However, Culha *et al*[88] disputed these findings and have shown that simultaneously occurring different etiologic factors may be responsible for the development of DM-related PE. These factors include ED (20.75%), chronic prostatitis (18.87%), depression (16.98%), anxiety (15.09%), FSH–LH abnormality (7.55%), hyperprolactinemia (7.55%), and hyperthyroidism (1.89%). These latter findings still await confirmation.

According to the consensus of the International Society for Sexual Medicine, acquired PE (*e.g.*, diabetes-related PE) is an ejaculation that always or nearly always occurs before or within about 3 min of vaginal penetration or, a significant and bothersome reduction in the latency time, associated with the incapability to delay ejaculation on all/nearly all vaginal penetrations, and negative personal consequences, as; distress, bother, frustration and/or avoidance of sexual intimacy[119]. Evaluation of diabetes-related PE includes a clinical history and careful physical examination focusing on all related symptoms, signs, and risk factors. A list of assessment steps that could be helpful in evaluating DM-related ejaculatory dysfunction is presented (Table 4). The most important dimensions in history taking include assessment of self-estimated IELT, subjective perceived control over ejaculation, existed distress by the condition, and the existence of an interpersonal difficulty owing to PE. Routine laboratory tests or neuro-physiological tests should merely directed by precise findings from either the history taking or physical examination[120].

In principle, scarce data are assessing the treatment of DM-related PE. Additionally, one should know that DM-related PE may be a heterogeneous group of patients. Hence, it is important to diagnose any associated comorbidity such as ED, depression, prostatitis, or hyperthyroidism as they should be treated first or at the same time as PE [120]. Therefore, the treatment may involve numerous interventions as per the kind of mechanism that would cause such condition: (1) The initial management for PE is controlling the patient's blood glucose that in some cases may allow recover the normal ejaculatory function[91,93]; (2) Amelioration of glycemic variability would improve PE in type 1 diabetes patients[96]; (3) In cases of concomitant ED and PE, ED should receive phosphodiesterase type 5 inhibitors before, or at least at the same time as, PE[91,93,94]. The efficacy of the combined use of phosphodiesterase type 5 inhibitors and dapoxetine in males with comorbid PE and ED are supported by some studies[121-123]; (4) DM-related PE patients had a worse response to 30-60 mg oral dapoxetine treatment compared to non-diabetic PE patients. Poor treatment outcomes in diabetes patients may be attributed to DM-associated complications[123]; and (5) Various behavioral techniques (such as 'squeeze' technique or 'stop-start' program) may be beneficial in those associated with psychological factors or those patients uncomfortable with pharmacological therapy. However, the long-term success of these maneuvers is limited[120].

DE and AE

DE (also termed retarded ejaculation, inhibited ejaculation (IE), inadequate ejaculation, male orgasmic disorder, or primary impotentia ejaculationis,) was used to describe "a marked delay in or inability to achieve ejaculation"[124]. Current diagnostic criteria from the Diagnostic and Statistical Manual of Mental Disorders, 5th edition, defines DE as either a marked delay in or a marked infrequency or absence of ejaculation on 75% to 100% of occasions for at least 6 mo of partnered sexual activity without the individual desiring delay and causing significant distress to the individual[125]. Therefore, the terminology DE aimed to describe all ejaculatory disorders that lead to a delay or absence of ejaculation (AE)[1]. Furthermore, the severity of DE was classified according to Kaplan criteria into mild and moderate forms and the most severe ones (AE or severe DE)[126]. For these reasons, both DE and AE will be discussed together in this section.

Overall, DE is the rarest and the least understood male sexual dysfunction. Its incidence in the general population rarely exceeds 3%[127,128]. There have been few

Table 4 Assessment steps in the evaluation of diabetes mellitus-related ejaculatory dysfunctions

History
Asking about the period from vaginal intromission to ejaculation (intravaginal ejaculatory latency time).
Is the patient unable to advance his ejaculatory response?
Is the patient or his partner distressed or bothered by the situation?
Is the symptom occurring since the first sexual experience or occurring after a period of normal ejaculatory performance?
Onset and duration of the symptom.
Is the symptom occurring on every/almost every attempt and with every partner?
Presence or absence of premonitory ejaculatory sensation.
The duration of thrusting before the suspension of intercourse.
Reasons for delay of intercourse (*e.g.*, fatigue, loss of erection, a sense of ejaculatory futility, or partner request).
Presence of post-coital self- or partner-assisted masturbation.
Psychogenic anejaculation/anorgasmia can be suspected when there is a history of nocturnal emission.
Patient's ability to get an erection, relax, sustain, and heighten sexual arousal.
Exclude anorgasmia by asking about lack of orgasm.
Whether orgasm is present but there is a lack of external ejaculation that may indicate retrograde ejaculate.
Feeling before ejaculation/orgasm: The inadequate combination of "friction and fantasy" may exacerbate DE.
Intercourse frequency.
Presence of other sexual dysfunctions such as ED (ability to initiate or maintain an erection), low libido.
Other symptoms of hypogonadism (such as lack of energy, depressed mood).
Masturbation habits
The life events/circumstances related to the complaint.
Sexual communication abilities.
Paraphilic inclinations/interests (may be related to DE and anejaculation).
Cultural or religious beliefs (if any).
History of a psychiatric disorder (may be the etiologic factor).
History of previous treatment for this symptom.
History of neurologic disorders, spinal cord injury, medical diseases, trauma, abdominal/pelvic operations, drug intake, or pelvic radiotherapy.
History of pelvic or testicular pain (may indicate inflammation).
History of dysuria, burning micturition, or any urinary symptom (indicate inflammation).
Clinical examination
Signs of diabetic complications and co-morbidities.
Signs of hypogonadism.
Rule out systemic disorders that contribute to ejaculation dysfunction as neurological impairment, endocrine/ urological diseases.
Examination for secondary sexual characteristics, penile and testicular abnormalities.
Examination of the epididymis, and vas deferens on each side.
PR examination to determine the prostate size, anal sphincter tone, and quality of the bulbocavernosus reflex.
The cremasteric reflex: measures intact L1-2 spinal segments, also mediating emission and psychogenic erection.
Perineal reflexes (bulbocavernosus and anal reflex) mediated by sacral segments, also mediating reflex erection (for intact S2–4 pathway).
Examination of pinprick and temperature sensations in the saddle area (perineal) and glans penis for healthy sacral cord segments.
Inability to feel testicular squeeze: measures the integrity of T11 to T12 spinal nerves *via* the sympathetic nervous system.
Examination of lower abdominal cutaneous reflex: measures intact Th11-12.
Penile biothesiometry.

Investigations
Blood levels of glucose, HbA1c, serum testosterone, thyrotropin, and prolactin to exclude other endocrine disorders.
Post-masturbation first-void urine if we suspect retrograde ejaculation to search for spermatozoa and fructose content to confirm retrograde ejaculate
Microbiological examination of expressed prostatic secretion and urine to verify or exclude associated genital infections.
Urine cytology to exclude bladder cancer
Serum prostate-specific antigen to exclude prostate cancer
Neurophysiologic investigations (bulbocavernosus evoked response and dorsal nerve somatosensory evoked-potentials): If there is clinical evidence of neurologic lesions. These tests are little used in clinical practice and usually do not affect management.
Trans-rectal ultrasound examination if we suspect ejaculatory duct obstruction, prostatic or seminal vesicle abnormalities or stones.
CT or MRI scans to assess pelvic anatomy if we suspect major pelvic lesions.

CT: Computed tomography; MRI: Magnetic resonance imaging.

attempts to study the prevalence of DE and AE in DM. While one study showed 0% prevalence among a series of 54 diabetes patients[129], another research demonstrated self-reported absent ejaculation and DE in 7.2 % and 0.36 % respectively among a total series of 276 diabetes patients[130]. However, sporadic cases were reported in literature either in the context of fertility evaluation[110,131] or as a sexual complaint [132]. It is thought that several pathophysiological factors may contribute to the development of DE and AE such as depression, progressive autonomic neuropathy, calcification of VD and SV, hypogonadism, or low sexual desire (Table 3).

Unfortunately, careful history is still the key there is no test to diagnose DE/AE [133]. It starts by excluding RE, genital tract obstruction, anorgasmia, and other sexual dysfunctions that may be misdiagnosed as DE/AE, such as ED, a subtly decreased libido, ejaculatory pain, the partner's sexual dysfunction, sexual orientation conflicts, or paraphilic inclinations/interests (Table 4). A focused psychosexual evaluation is critical and typically begins by differentiating whether the complaint concerns DE/AE, the sensation of orgasm, or both. Attention should be given to identifying reversible factors such as poor glycemic control, hypogonadism low sexual desire, psychological factors, and genital infections. A focused clinical examination, laboratory tests, as well as radiologic imaging may help to diagnose these risk factors (Table 4). Sometimes the diagnosis of those risk factor(s) unfolds over time many clinical visits[134].

In this context, defining solid approaches for the treatment of DM-related DE/AE is difficult for a condition that the literature is restricted to case reports, case series, or small studies. It is assumed that intensive glycemic control may reduce the prevalence of diabetic autonomic dysfunction and might slow the deterioration of DM-related DE/AE; unfortunately, no article studying this effect has been published. If organic and iatrogenic reasons have been let off in DM-related DE/AE behavioral therapy may be considered[1]. Several drugs are recognized for the possible use in DE/AE. Those agents include; testosterone, amantadine, cyproheptadine, cabergoline, bupropion, yohimbine, buspirone, bethanechol, and others. Yet, no drug was approved for this indication[125]. Testosterone replacement therapy may be an appropriate option for DM-related DE/AE with decreased testosterone levels. However, in a clinical trial, in which diabetes is not an exclusion criterion, Paduch *et al*[82] reported that treatment of testosterone-deficient DE patients with a 2% solution of testosterone is not linked to improved perceived delay of ejaculation suggesting that testosterone deficiency is not the sole contributor to DE. Mechanistically, diabetic autonomic neuropathy is an important factor in the development of DM-related DE/AE that may affect neural systems at all levels of emission and ejection. Once diabetic autonomic neuropathy becomes clinically evident, there is no treatment to reverse it[135]. In the most severe cases, there may be a total lack of seminal emission leading to male infertility. If infertility is an issue, several approaches can be employed: (1) Assisted ejaculation by penile vibratory stimulation (PVS) is an option for sperm retrieval[136] because it is noninvasive and inexpensive[137]. However, DM patients frequently fail to obtain sperms by this method because it requires intact lumbosacral pathways[136,137]; (2) In cases in whom PVS fails, electroejaculation (EEJ) may be effective to retrieve spermatozoa[99,138]; and (3) If these procedures fail, surgical sperm retrieval can be tried through VD aspiration[139], percutaneous epididymal sperm aspiration (PESA) [140], or testicular sperm extraction (TESA)[141]. Using spermatozoa obtained by any of these procedures and utilizing intracytoplasmic sperm injection (ICSI), one can

attain clinical pregnancy and live birth rates in DM-related AE. However, the fertilization rate and high-quality embryo rate in diabetic PE patients are lower than in non-diabetic PE patients[140].

RE

RE is unique in that it is almost exclusively organic in origin. Despite being a common type of ejaculatory dysfunction, it is responsible for only 0.3%–2% of infertility[142,143]. Specifically, young men with type 1 diabetes have a higher incidence of ejaculatory dysfunction estimated to be 5%-18% of cases due to diabetic neuropathy, which has been shown to cause male infertility[29,144,145].

RE is an ejaculation that is deposited into the posterior urethra but propelled backward into the urinary bladder. In the normal state, the bladder neck closes with high pressure under the sympathetic control during orgasm and the seminal bolus takes the route of least resistance, being antegrade. Diabetic neuropathy can interfere with the sympathetic fibers that provide for normal high-pressure bladder neck closure, causing a comparatively low-pressure route into the urinary bladder for semen[146].

Impairment of sympathetic innervation of the urinary bladder neck was supposed to be a cause of diabetic RE. Ibragimov *et al*[147] used the liquid profilometric technique to examine 3 groups of men: 8 patients with RE; 5 patients with DM without ejaculatory disorders; and 7 healthy subjects. Diabetic RE patients showed no elevation of the intraureteral pressure in the area of the inner sphincter of the urinary bladder, which evidenced its atony. In health the elevation of vesical pressure is usually accompanied by increased ureteral resistance, thus maintaining the stability of the positive pressure gradient and preventing the escape of urine. Correlation analysis revealed alterations of the interrelations between both intravesical and sphincter pressures in diabetes patients evidencing the disorders of somatic innervation of the outer ureteral sphincter being more pronounced in these patients.

DIAGNOSIS

RE can be partial or complete and the diagnosis is frequently suggested by the patient's report of cloudy urine following orgasm confirmed by a post-ejaculatory urine analysis that reveals sperm, seminal fluid, or fructose[148]. McMahon[149] endorsed that the post-orgasmic urine should be centrifuged and visualizing of 10-15 sperm/high-power field could confirm RE diagnosis whereas Fedder *et al*[150] defined RE as > 1 million sperms in a post-ejaculatory urine sample.

TREATMENT STRATEGIES

Infertility is usually the main concern in RE patients as the combination of dry orgasm and infertility makes the condition upsetting to the patient and his partner[151]. Therefore, many lines of therapeutic approaches were advocated, either medical or surgical, with limited success rates.

Medical treatment

It is based either on increasing the sympathetic tone of the urinary bladder or on decreasing the parasympathetic activity but the onset of side effects and the lack of response should be considered. Several treatments were proposed with varied results [152-161] (Table 5).

Endourethral collagen injection

Kurbatov *et al*[145] analyzed the long-term outcome of endourethral injection of volume-forming material (VFM) of collagen type 2 into the bladder neck submucosa in 23 patients with RE secondary to type 1 DM with complete RE refractory to imipramine. These patients were randomized with a 1: 1 ratio into 2 groups; group A (endourethral collage type 2 injection) and group B (endourethral saline water injection). This technique included an endoscopic injection of VFM such as collagen into bladder neck submucosa. In group A, significant differences from baseline to 12 mo were detected relative to antegrade volume (mean difference: 0.71 mL), antegrade count (mean difference: 45.6 million/mL), antegrade total sperm motility (mean difference: 15.4%), and antegrade progressive sperm motility (mean difference: 8.4%).

Table 5 Studies involving medical treatment for reversal of diabetic retrograde ejaculation

Ref.	Dosage	Ejaculation after	*n*	No. of successes	Ejaculate volume (ml)	Sperm count (10^6/m)	Sperm motility (%)
Brompheniramine							
Andaloro *et al*[152]	16 mg/d p.o.	12 h	1	1	Unclear	Unclear	Unclear
Budd[153]	16 mg/d p.o.	3 d	1	1	Unclear	Unclear	Unclear
Chlorpheniramine + phenylpropanalamine							
Stewart *et al*[154]	50 mg/d p.o.	Unclear	1	1	4.5	Normal	Normal
Ephedrine							
Gilja *et al*[155]	50 mg/d p.o.	4 wk	17	3	Unclear	Unclear	Unclear
Arafa *et al*[156]	120 mg twice/d	14 d	23	11	Unclear	Unclear	Unclear
Shoshany *et al*[157]	60 mg/6 h the day before test + 2 doses on test day	At the test	6	4	1.5	Unclear	17.8
Imipramine							
Brooks *et al*[158]	75 mg/d p.o.	1 wk	2	2	3	1.72	33
Okada *et al*[159]	25-150 mg/d	Unclear	7	3	Unclear	Unclear	Unclear
Gilja *et al*[155]	75 mg/d p.o.	4 wk	14	2	Unclear	Unclear	Unclear
Eppel *et al*[160]	50 mg/d p.o.	5 d	3	3	8	20	50
Arafa *et al*[156]	50 mg/d p.o	14 d	23	10	Unclear	Unclear	Unclear
Imramine + pseudoepherine							
Arafa *et al*[156]	50 + 120 mg/d	14 d	23	16	Unclear	Unclear	Unclear
Amoxapine							
Hibi *et al*[161]	50 mg/d	1 mo	1	1	0.2	213	53

It was concluded that correcting RE in type 1 DM patients could be accomplished with the endourethral injection of collagen type 2.

SPERM RETRIEVAL

Beyond using standard sperm retrieval techniques such as; TESE and PESA, 3 methods of sperm retrieval were recognized for managing infertility in RE patients. These techniques include; centrifugation and resuspension of post-ejaculatory urine specimens, the Hotchkiss (or modified Hotchkiss) technique, as well as ejaculation on a full urinary bladder.

Centrifugation and resuspension: To improve the conditions for the sperm, the patient is asked to either increase their fluid intake or to take sodium bicarbonate to dilute or alkalize the urine. A post-orgasmic urine sample is collected by either introducing a catheter or spontaneous voiding. This sample is centrifuged and suspended in a medium such as bovine serum albumin, human serum albumin, Earle's/Hank's, phosphate-buffered medium. The resultant modified sperm mixture can be used in assisted reproductive techniques (ART) (Table 6)[162-165]. In their meta-analysis in couples with the male partner with RE, Jefferys *et al*[142] reported a 15% pregnancy rate/cycle (0%–100%) after using the centrifugation and resuspension method.

Hotchkiss method: It involves emptying the urinary bladder before ejaculation by a catheter, washing out and instilling a small quantity of lactated Ringers to improve the ambient conditions of the bladder. The patient then ejaculates and the semen is retrieved by catheterization or voiding[166].

Modified Hotchkiss method: It involves a variance in the instillation medium. Pregnancy rates were 24%/cycle (0%–100%)[142]. Philippon *et al*[167] reported the largest series of births using frozen-thawed sperms retrieved from post-ejaculatory urine by a this technique that allows for successful association with sperm cryopreser-

Table 6 Semen parameters of studies recovering sperms from alkalized urine in diabetic premature ejaculation patients

Ref.	Medium installed	Post-masturbatory retrieval	No. of patients	Total sperm count (10^6)	Total sperm motility (%)	Pregnancies
Brassesco *et al* [163]	$NaHCO_3$ 4 g	Voiding	3	91	28	3
Templeton *et al* [162]	$NaHCO_3$	Voiding	1	Unclear	2–21	0
Shangold *et al*[164]	$NaHCO_3$ 1.6 g	Voiding	1	30–240	0	5

vation, leading to efficient management of couples with refractory RE with an average live birth rate/transfer of 28%.

Ejaculation on a full bladder: The patient is encouraged to ejaculate on a full urinary bladder and semen is suspended in Baker's buffer[162].

EEJ

EEJ has been used to a restricted degree in diabetic men who have developed ejaculatory failure as a consequence of diabetic neuropathy. Gerig *et al*[131] described the experience of 2 male fertility programs using EEJ in managing men with ejaculatory failure secondary to DM. Overall, 29 EEJ procedures were performed in seven diabetic men with ejaculatory failure. Following EEJ, retrograde semen specimens retrieved from the urinary bladder contained a mean of 3444.5 million sperm (range 269.2-4996 million), mean sperm motility was 4% (range 0%-11%). Semen specimens were used for intrauterine insemination. It was concluded that EEJ can be successfully used to retrieve sperms from men with ejaculatory failure due to DM. That procedure requires general anesthesia, and the pregnancy rates after intrauterine insemination with the processed sperm were low. Therefore, AET could offer a practicable alternate, yielding higher success rates.

ICSI

ICSI-ART can greatly reduce the impact of sperm factors of infertility[167]. In cases of diabetic RE, TESA is combined with ICSI to treat infertility. Liu *et al*[168] assessed the effect of TESA-ICSI on first cycle ICSI-embryo transfer for type 2 diabetic patients in 1219 azoospermic patients or RE who were treated with TESA-ICSI classified into 2 groups; type 2 DM group (n = 54) and non-diabetic controls (n = 1165). There were no significant differences in clinical pregnancy, implantation, normal fertilization, or cleavage rates between these groups.

CONCLUSION

Ejaculatory dysfunction encompasses several disorders related to DM and its complications, such as PE, DE, AE, RE, ejaculatory pain, anesthetic ejaculation, decreased ejaculate volume, and decreased force of ejaculation. All these disorders should be looked for thoroughly during the clinical evaluation of diabetic men. Besides, introducing the suitable option and/or maneuvers to treat these disorders should be tailored according to each case.

REFERENCES

1 **Rowland D**, McMahon CG, Abdo C, Chen J, Jannini E, Waldinger MD, Ahn TY. Disorders of orgasm and ejaculation in men. *J Sex Med* 2010; **7**: 1668-1686 [PMID: 20388164 DOI: 10.1111/j.1743-6109.2010.01782.x]

2 **Andersson KE**, Abdel-Hamid IA. Therapeutic targets for premature ejaculation. *Maturitas* 2011; **70**: 26-33 [PMID: 21816550 DOI: 10.1016/j.maturitas.2011.06.007]

3 **Parnham A**, Serefoglu EC. Retrograde ejaculation, painful ejaculation and hematospermia. *Transl Androl Urol* 2016; **5**: 592-601 [PMID: 27652230 DOI: 10.21037/tau.2016.06.05]

4 **Phillips E**, Carpenter C, Oates RD. Ejaculatory dysfunction. *Urol Clin North Am* 2014; **41**: 115-128 [PMID: 24286771 DOI: 10.1016/j.ucl.2013.08.018]

5 **Clement P**, Giuliano F. Physiology and Pharmacology of Ejaculation. *Basic Clin Pharmacol Toxicol* 2016; **119** Suppl 3: 18-25 [PMID: 26709195 DOI: 10.1111/bcpt.12546]

6 **Everaert K**, de Waard WI, Van Hoof T, Kiekens C, Mulliez T, D'herde C. Neuroanatomy and neurophysiology related to sexual dysfunction in male neurogenic patients with lesions to the spinal cord or peripheral nerves. *Spinal Cord* 2010; **48**: 182-191 [PMID: 20048757 DOI: 10.1038/sc.2009.172]

7 **Aumüller G**, Riva A. Morphology and functions of the human seminal vesicle. *Andrologia* 1992; **24**: 183-196 [PMID: 1642333 DOI: 10.1111/j.1439-0272.1992.tb02636.x]

8 **Witte LP**, Chapple CR, de la Rosette JJ, Michel MC. Cholinergic innervation and muscarinic receptors in the human prostate. *Eur Urol* 2008; **54**: 326-334 [PMID: 18160204 DOI: 10.1016/j.eururo.2007.12.007]

9 **Comarr AE**. Sexual function among patients with spinal cord injury. *Urol Int* 1970; **25**: 134-168 [PMID: 5422714 DOI: 10.1159/000279669]

10 **Gerstenberg TC**, Levin RJ, Wagner G. Erection and ejaculation in man. Assessment of the electromyographic activity of the bulbocavernosus and ischiocavernosus muscles. *Br J Urol* 1990; **65**: 395-402 [PMID: 2340374 DOI: 10.1111/j.1464-410x.1990.tb14764.x]

11 **Onuf B**. Notes on the arrangement and function of the cell groups in the sacral region of the spinal cord. *J Nerv Ment Dis* 1899; **26**: 498-504

12 **Truitt WA**, Coolen LM. Identification of a potential ejaculation generator in the spinal cord. *Science* 2002; **297**: 1566-1569 [PMID: 12202834 DOI: 10.1126/science.1073885]

13 **Staudt MD**, Truitt WA, McKenna KE, de Oliveira CV, Lehman MN, Coolen LM. A pivotal role of lumbar spinothalamic cells in the regulation of ejaculation *via* intraspinal connections. *J Sex Med* 2012; **9**: 2256-2265 [PMID: 22189051 DOI: 10.1111/j.1743-6109.2011.02574.x]

14 **Chéhensse C**, Facchinetti P, Bahrami S, Andrey P, Soler JM, Chrétien F, Bernabé J, Clément P, Denys P, Giuliano F. Human spinal ejaculation generator. *Ann Neurol* 2017; **81**: 35-45 [PMID: 27917533 DOI: 10.1002/ana.24819]

15 **Corona G**, Jannini EA, Vignozzi L, Rastrelli G, Maggi M. The hormonal control of ejaculation. *Nat Rev Urol* 2012; **9**: 508-519 [PMID: 22869001 DOI: 10.1038/nrurol.2012.147]

16 **Stadler B**, Whittaker MR, Exintaris B, Middendorff R. Oxytocin in the Male Reproductive Tract; The Therapeutic Potential of Oxytocin-Agonists and-Antagonists. *Front Endocrinol (Lausanne)* 2020; **11**: 565731 [PMID: 33193084 DOI: 10.3389/fendo.2020.565731]

17 **Vignozzi L**, Filippi S, Morelli A, Luconi M, Jannini E, Forti G, Maggi M. Regulation of epididymal contractility during semen emission, the first part of the ejaculatory process: a role for estrogen. *J Sex Med* 2008; **5**: 2010-6; quiz 2017 [PMID: 18783525 DOI: 10.1111/j.1743-6109.2008.00914.x]

18 **Krüger TH**, Haake P, Chereath D, Knapp W, Janssen OE, Exton MS, Schedlowski M, Hartmann U. Specificity of the neuroendocrine response to orgasm during sexual arousal in men. *J Endocrinol* 2003; **177**: 57-64 [PMID: 12697037 DOI: 10.1677/joe.0.1770057]

19 **Krüger TH**, Hartmann U, Schedlowski M. Prolactinergic and dopaminergic mechanisms underlying sexual arousal and orgasm in humans. *World J Urol* 2005; **23**: 130-138 [PMID: 15889301 DOI: 10.1007/s00345-004-0496-7]

20 **Rastrelli G**, Corona G, Maggi M. Testosterone and sexual function in men. *Maturitas* 2018; **112**: 46-52 [PMID: 29704917 DOI: 10.1016/j.maturitas.2018.04.004]

21 **Keast JR**. Effects of testosterone on pelvic autonomic pathways: progress and pitfalls. *J Auton Nerv Syst* 2000; **79**: 67-73 [PMID: 10699636 DOI: 10.1016/s0165-1838(99)00087-9]

22 **Ye F**, McCoy SC, Ross HH, Bernardo JA, Beharry AW, Senf SM, Judge AR, Beck DT, Conover CF, Cannady DF, Smith BK, Yarrow JF, Borst SE. Transcriptional regulation of myotrophic actions by testosterone and trenbolone on androgen-responsive muscle. *Steroids* 2014; **87**: 59-66 [PMID: 24928725 DOI: 10.1016/j.steroids.2014.05.024]

23 **Gupta N**, Carvajal M, Jurewicz M, Gilbert BR. Bulbocavernosus muscle area as a novel marker for hypogonadism. *Asian J Urol* 2017; **4**: 3-9 [PMID: 29264199 DOI: 10.1016/j.ajur.2016.11.002]

24 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013; **36**: S67-74 [PMID: 23264425 DOI: 10.2337/dc13-S067]

25 **Ellenberg M**, Weber H. Retrograde ejaculation in diabetic neuropathy. *Ann Intern Med* 1966; **65**: 1237-1246 [PMID: 5928483 DOI: 10.7326/0003-4819-65-6-1237]

26 **Haddad WT Jr**, Moritsugu OT, Fornazari VR, Neto CAFC, da Frota YB, Molina CAF, Elias J Jr, Pazin-Filho A, Muglia VF. Prevalence of vas deferens calcifications on abdominal CT examinations and association with systemic conditions. *AJR Am J Roentgenol* 2020; **215**: 1398-1402 [PMID: 33021834 DOI: 10.2214/AJR.19.22672]

27 **La Vignera S**, Condorelli RA, Vicari E, Lotti F, Favilla V, Morgia G, Maggi M, Calogero AE. Seminal vesicles and diabetic neuropathy: ultrasound evaluation after prolonged treatment with a selective phosphodiesterase-5 inhibitor. *Andrology* 2013; **1**: 245-250 [PMID: 23413137 DOI: 10.1111/j.2047-2927.2012.00025.x]

28 **La Vignera S**, Condorelli RA, Di Mauro M, Lo Presti D, Mongioì LM, Russo G, Calogero AE. Reproductive function in male patients with type 1 diabetes mellitus. *Andrology* 2015; **3**: 1082-1087 [PMID: 26446574 DOI: 10.1111/andr.12097]

29 **Hylmarova S**, Stechova K, Pavlinkova G, Peknicova J, Macek M, Kvapil M. The impact of type 1 diabetes mellitus on male sexual functions and sex hormone levels. *Endocr J* 2020; **67**: 59-71 [PMID: 31619592 DOI: 10.1507/endocrj.EJ19-0280]

30 **Gaunay G**, Nagler HM, Stember DS. Reproductive sequelae of diabetes in male patients. *Endocrinol Metab Clin North Am* 2013; **42**: 899-914 [PMID: 24286955 DOI: 10.1016/j.ecl.2013.07.003]

31 **Sachs BD**, Baum MJ, Melman A. Normal sexual behavior and penile reflexes in long-term diabetic male rats. *Arch Androl* 1982; **9**: 351-353 [PMID: 7165414 DOI: 10.3109/01485018208990261]

32 **Clark JT**. Sexual function in altered physiological states: comparison of effects of hypertension, diabetes, hyperprolactinemia, and others to "normal" aging in male rats. *Neurosci Biobehav Rev* 1995; **19**: 279-302 [PMID: 7630583 DOI: 10.1016/0149-7634(94)00058-9]

33 **Scarano WR**, Messias AG, Oliva SU, Klinefelter GR, Kempinas WG. Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. *Int J Androl* 2006; **29**: 482-488 [PMID: 16524366 DOI: 10.1111/j.1365-2605.2006.00682.x]

34 **Steger RW**, Kienast SG. Effect of continuous versus delayed insulin replacement on sex behavior and neuroendocrine function in diabetic male rats. *Diabetes* 1990; **39**: 942-948 [PMID: 1973672 DOI: 10.2337/diab.39.8.942]

35 **Murray FT**, Johnson RD, Sciadini M, Katovich MJ, Rountree J, Jewett H. Erectile and copulatory dysfunction in chronically diabetic BB/WOR rats. *Am J Physiol* 1992; **263**: E151-E157 [PMID: 1636693 DOI: 10.1152/ajpendo.1992.263.1.E151]

36 **McVary KT**, Rathnau CH, McKenna KE. Sexual dysfunction in the diabetic BB/WOR rat: a role of central neuropathy. *Am J Physiol* 1997; **272**: R259-R267 [PMID: 9039017 DOI: 10.1152/ajpregu.1997.272.1.R259]

37 **Ebiko M**, Yonezawa A, Yoshuzumi M. Changes in ejaculatory capacity in the type I diabetic rats. *Jpn J Sex Med* 2006; **21**: 221-232

38 **Yonezawa A**, Ebiko M, Yoshizumi M, Ise SN, Watanabe C, Mizoguchi H, Iwasaki M, Kimura Y, Sakurada S. Effects of insulin replacement on ejaculatory dysfunction in streptozotocin-induced diabetic rats. *Int J Urol* 2009; **16**: 208-211 [PMID: 19183231 DOI: 10.1111/j.1442-2042.2008.02214.x]

39 **Suresh S**, Prakash S. Effect of Mucuna pruriens (Linn.) on sexual behavior and sperm parameters in streptozotocin-induced diabetic male rat. *J Sex Med* 2012; **9**: 3066-3078 [PMID: 20456630 DOI: 10.1111/j.1743-6109.2010.01831.x]

40 **De A**, Singh MF, Singh V, Ram V, Bisht S. Treatment effect of l-Norvaline on the sexual performance of male rats with streptozotocin induced diabetes. *Eur J Pharmacol* 2016; **771**: 247-254 [PMID: 26671005 DOI: 10.1016/j.ejphar.2015.12.008]

41 **Shi GJ**, Zheng J, Wu J, Qiao HQ, Chang Q, Niu Y, Sun T, Li YX, Yu JQ. Protective effects of Lycium barbarum polysaccharide on male sexual dysfunction and fertility impairments by activating hypothalamic pituitary gonadal axis in streptozotocin-induced type-1 diabetic male mice. *Endocr J* 2017; **64**: 907-922 [PMID: 28794341 DOI: 10.1507/endocrj.EJ16-0430]

42 **Li ZM**, Liu N, Jiang YP, Yang JM, Zheng J, Sun M, Li YX, Sun T, Wu J, Yu JQ. Vitexin alleviates streptozotocin-induced sexual dysfunction and fertility impairments in male mice *via* modulating the hypothalamus-pituitary-gonadal axis. *Chem Biol Interact* 2019; **297**: 119-129 [PMID: 30365938 DOI: 10.1016/j.cbi.2018.10.013]

43 **Lert-Amornpat T**, Maketon C, Fungfuang W. Effect of Kaempferia parviflora on sexual performance in streptozotocin-induced diabetic male rats. *Andrologia* 2017; **49** [PMID: 28295466 DOI: 10.1111/and.12770]

44 **Fernández-Collazo EL**, Foglia VG. Sexual behavior of the male diabetic rat. *Physiol Behav* 1970; **5**: 1451-1454 [PMID: 5524534 DOI: 10.1016/0031-9384(70)90135-6]

45 **Hassan AA**, Hassouna MM, Taketo T, Gagnon C, Elhilali MM. The effect of diabetes on sexual behavior and reproductive tract function in male rats. *J Urol* 1993; **149**: 148-154 [PMID: 8417201 DOI: 10.1016/s0022-5347(17)36028-7]

46 **Pontes DA**, Fernandes GS, Piffer RC, Gerardin DC, Pereira OC, Kempinas WG. Ejaculatory dysfunction in streptozotocin-induced diabetic rats: the role of testosterone. *Pharmacol Rep* 2011; **63**: 130-138 [PMID: 21441620 DOI: 10.1016/s1734-1140(11)70407-8]

47 **Ghaheri M**, Miraghaee S, Babaei A, Mohammadi B, Kahrizi D, Saivosh Haghighi ZM, Bahrami G. Effect of Stevia rebaudiana Bertoni extract on sexual dysfunction in Streptozotocin-induced diabetic male rats. *Cell Mol Biol (Noisy-le-grand)* 2018; **64**: 6-10 [PMID: 29433621 DOI: 10.14715/cmb/2018.64.2.2]

48 **Minaz N**, Razdan R, Hammock BD, Mujwar S, Goswami SK. Impact of diabetes on male sexual function in streptozotocin-induced diabetic rats: Protective role of soluble epoxide hydrolase inhibitor. *Biomed Pharmacother* 2019; **115**: 108897 [PMID: 31102913 DOI: 10.1016/j.biopha.2019.108897]

49 **Yagihashi S**, Sima AA. Diabetic autonomic neuropathy in the BB rat. Ultrastructural and morphometric changes in sympathetic nerves. *Diabetes* 1985; **34**: 558-564 [PMID: 4007283 DOI: 10.2337/diab.34.6.558]

50 **Tomlinson DR**, Gillon KR, Smith MG. Axonal transport of noradrenaline and noradrenergic transmission in rats with streptozotocin-induced diabetes. *Diabetologia* 1982; **22**: 199-204 [PMID: 6176490 DOI: 10.1007/BF00283753]

51 **Longhurst PA**, Brotcke TP, Burrell CL, Belis JA. Comparison of the effects of castration and streptozotocin-induced diabetes mellitus on contractile responses of the rat vas deferens. *Pharmacology* 1989; **38**: 253-262 [PMID: 2755965 DOI: 10.1159/000138544]

52 **Kaschube M**, Möller-Hartmann H, Zetler G. The field-stimulated vas deferens of the streptozotocin-diabetic mouse: effects of prazosin, alpha, beta-methylene ATP, and variation of stimulation parameters. *J Neural Transm* 1989; **77**: 171-180 [PMID: 2760603 DOI: 10.1007/BF01248930]

53 **Kamata K**, Kirisawa H. Changes in electrophysiological properties and noradrenaline response in

vas deferens of diabetic rats. *Eur J Pharmacol* 1998; **350**: 237-241 [PMID: 9696413 DOI: 10.1016/s0014-2999(98)00271-4]

54 **Güneş A**, Ceylan A, Sarioglu Y, Stefek M, Bauer V, Karasu C; Antioxidants in Diabetes-induced Complications (ADIC) Study Group. Reactive oxygen species mediate abnormal contractile response to sympathetic nerve stimulation and noradrenaline in the vas deferens of chronically diabetic rats: effects of in vivo treatment with antioxidants. *Fundam Clin Pharmacol* 2005; **19**: 73-79 [PMID: 15660962 DOI: 10.1111/j.1472-8206.2004.00312.x]

55 **Tsounapi P**, Honda M, Dimitriadis F, Shimizu S, Shiomi T, Hikita K, Saito M, Tomita S, Sofikitis N, Takenaka A. Antioxidant treatment ameliorates diabetes-induced dysfunction of the vas deferens in a rat model. *Andrologia* 2018; **50** [PMID: 28224697 DOI: 10.1111/and.12795]

56 **Sakai Y**, Honda H. Hyperactivity of Ca channels in vasa deferentia smooth muscle of diabetic rats. *Pharmacol Biochem Behav* 1987; **27**: 227-229 [PMID: 2442778 DOI: 10.1016/0091-3057(87)90562-4]

57 **Sakai Y**, Aihara K, Honda H, Inazu M. Calcium mobilization and phosphatidylinositol turnover in vas deferens smooth muscle of diabetic rats. *Eur J Pharmacol* 1989; **162**: 475-481 [PMID: 2744081 DOI: 10.1016/0014-2999(89)90338-5]

58 **Elbadawi A**. Ultrastructure of vesicourethral innervation. II. Postganglionic axoaxonal synapses in intrinsic innervation of the vesicourethral lissosphincter: a new structural and functional concept in micturition. *J Urol* 1984; **131**: 781-790 [PMID: 6708203 DOI: 10.1016/s0022-5347(17)50624-2]

59 **Fowler CJ**, Griffiths D, de Groat WC. The neural control of micturition. *Nat Rev Neurosci* 2008; **9**: 453-466 [PMID: 18490916 DOI: 10.1038/nrn2401]

60 **Torimoto K**, Hirao Y, Matsuyoshi H, de Groat WC, Chancellor MB, Yoshimura N. alpha1-Adrenergic mechanism in diabetic urethral dysfunction in rats. *J Urol* 2005; **173**: 1027-1032 [PMID: 15711370 DOI: 10.1097/01.ju.0000146268.45662.36]

61 **Sandrini M**, Vitale G, Vergoni AV, Ottani A, Bertolini A. Streptozotocin-induced diabetes provokes changes in serotonin concentration and on 5-HT1A and 5-HT2 receptors in the rat brain. *Life Sci* 1997; **60**: 1393-1397 [PMID: 9096260 DOI: 10.1016/s0024-3205(97)00084-2]

62 **Abraham PM**, Anju TR, Jayanarayanan S, Paulose CS. Serotonergic receptor upregulation in cerebral cortex and down regulation in brainstem of streptozotocin induced diabetic rats: antagonism by pyridoxine and insulin. *Neurosci Lett* 2010; **483**: 23-27 [PMID: 20655360 DOI: 10.1016/j.neulet.2010.07.042]

63 **Martin-Tuite P**, Shindel AW. Management options for premature ejaculation and delayed ejaculation in men. *Sex Med Rev* 2020; **8**: 473-485 [PMID: 31668585 DOI: 10.1016/j.sxmr.2019.09.002]

64 **Kühn-Velten N**, Waldenburger D, Staib W. Evaluation of steroid biosynthetic lesions in isolated Leydig cells from the testes of streptozotocin-diabetic rats. *Diabetologia* 1982; **23**: 529-533 [PMID: 6295863 DOI: 10.1007/BF00254305]

65 **Anderson JE**, Thliveris JA. Morphometry and cytochemistry of Leydig cells in experimental diabetes. *Am J Anat* 1987; **180**: 41-48 [PMID: 3310596 DOI: 10.1002/aja.1001800104]

66 **Ricci G**, Catizone A, Esposito R, Pisanti FA, Vietri MT, Galdieri M. Diabetic rat testes: morphological and functional alterations. *Andrologia* 2009; **41**: 361-368 [PMID: 19891634 DOI: 10.1111/j.1439-0272.2009.00937.x]

67 **Murray FT**, Cameron DF, Orth JM, Katovich MJ. Gonadal dysfunction in the spontaneously diabetic BB rat: alterations of testes morphology, serum testosterone and LH. *Horm Metab Res* 1985; **17**: 495-501 [PMID: 4065811 DOI: 10.1055/s-2007-1013588]

68 **Cameron DF**, Rountree J, Schultz RE, Repetta D, Murray FT. Sustained hyperglycemia results in testicular dysfunction and reduced fertility potential in BBWOR diabetic rats. *Am J Physiol* 1990; **259**: E881-E889 [PMID: 2260654 DOI: 10.1152/ajpendo.1990.259.6.E881]

69 **Ohta T**, Katsuda Y, Miyajima K, Sasase T, Kimura S, Tong B, Yamada T. Gender differences in metabolic disorders and related diseases in spontaneously diabetic Torii-Lepr(fa) rats. *J Diabetes Res* 2014; **2014**: 841957 [PMID: 24892034 DOI: 10.1155/2014/841957]

70 **Nakane Y**, Kemmochi Y, Ogawa N, Sasase T, Ohta T, Higami Y, Fukai F. Hyperglycemia contributes to the development of Leydig cell hyperplasia in male Spontaneously Diabetic Torii rats. *J Toxicol Pathol* 2020; **33**: 121-129 [PMID: 32425345 DOI: 10.1293/tox.2019-0088]

71 **Seethalakshmi L**, Menon M, Diamond D. The effect of streptozotocin-induced diabetes on the neuroendocrine-male reproductive tract axis of the adult rat. *J Urol* 1987; **138**: 190-194 [PMID: 3599211 DOI: 10.1016/s0022-5347(17)43042-4]

72 **Wolfe A**, Divall S, Wu S. The regulation of reproductive neuroendocrine function by insulin and insulin-like growth factor-1 (IGF-1). *Front Neuroendocrinol* 2014; **35**: 558-572 [PMID: 24929098 DOI: 10.1016/j.yfrne.2014.05.007]

73 **Oksanen A**. Testicular lesions of streptozotocin diabetic rats. *Horm Res* 1975; **6**: 138-144 [PMID: 130334 DOI: 10.1159/000178671]

74 **Sudha S**, Valli G, Julie PM, Arunakaran J, Govindarajulu P, Balasubramanian K. Influence of streptozotocin-induced diabetes and insulin treatment on the pituitary-testicular axis during sexual maturation in rats. *Exp Clin Endocrinol Diabetes* 2000; **108**: 14-20 [PMID: 10768827 DOI: 10.1055/s-0032-1329210]

75 **Ballester J**, Muñoz MC, Domínguez J, Rigau T, Guinovart JJ, Rodríguez-Gil JE. Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. *J Androl* 2004; **25**: 706-719 [PMID: 15292100 DOI: 10.1002/j.1939-4640.2004.tb02845.x]

76 **Neirijnck Y**, Calvel P, Kilcoyne KR, Kühne F, Stévant I, Griffeth RJ, Pitetti JL, Andric SA, Hu MC, Pralong F, Smith LB, Nef S. Insulin and IGF1 receptors are essential for the development and steroidogenic function of adult Leydig cells. *FASEB J* 2018; **32**: 3321-3335 [PMID: 29401624 DOI: 10.1096/fj.201700769RR]

77 **Steger RW**. Testosterone replacement fails to reverse the adverse effects of streptozotocin-induced diabetes on sexual behavior in the male rat. *Pharmacol Biochem Behav* 1990; **35**: 577-582 [PMID: 2140195 DOI: 10.1016/0091-3057(90)90293-q]

78 **Nutsch VL**, Will RG, Tobiansky DJ, Reilly MP, Gore AC, Dominguez JM. Age-related changes in sexual function and steroid-hormone receptors in the medial preoptic area of male rats. *Horm Behav* 2017; **96**: 4-12 [PMID: 28882473 DOI: 10.1016/j.yhbeh.2017.08.008]

79 **Murashima A**, Kishigami S, Thomson A, Yamada G. Androgens and mammalian male reproductive tract development. *Biochim Biophys Acta* 2015; **1849**: 163-170 [PMID: 24875095 DOI: 10.1016/j.bbagrm.2014.05.020]

80 **Dabaja AA**, Wosnitzer MS, Mielnik A, Bolyakov A, Schlegel PN, Paduch DA. Bulbocavernosus muscle area measurement: a novel method to assess androgenic activity. *Asian J Androl* 2014; **16**: 618-622 [PMID: 24589463 DOI: 10.4103/1008-682X.123681]

81 **Corona G**, Jannini EA, Mannucci E, Fisher AD, Lotti F, Petrone L, Balercia G, Bandini E, Chiarini V, Forti G, Maggi M. Different testosterone levels are associated with ejaculatory dysfunction. *J Sex Med* 2008; **5**: 1991-1998 [PMID: 18399946 DOI: 10.1111/j.1743-6109.2008.00803.x]

82 **Paduch DA**, Polzer PK, Ni X, Basaria S. Testosterone replacement in androgen-deficient men with ejaculatory dysfunction: A randomized controlled trial. *J Clin Endocrinol Metab* 2015; **100**: 2956-2962 [PMID: 26158605 DOI: 10.1210/jc.2014-4434]

83 **Damassa DA**, Smith ER, Tennent B, Davidson JM. The relationship between circulating testosterone levels and male sexual behavior in rats. *Horm Behav* 1977; **8**: 275-286 [PMID: 881168 DOI: 10.1016/0018-506x(77)90002-2]

84 **Saenz de Tejada I**, Goldstein I. Diabetic penile neuropathy. *Urol Clin North Am* 1988; **15**: 17-22 [PMID: 2830692]

85 **Steger RW**, Amador A, Lam E, Rathert J, Weis J, Smith MS. Streptozotocin-induced deficits in sex behavior and neuroendocrine function in male rats. *Endocrinology* 1989; **124**: 1737-1743 [PMID: 2522388 DOI: 10.1210/endo-124-4-1737]

86 **Al-Roujayee A**. Improvement of sexual behavior, sperm quantity and quality by Quercetin in streptozotocin-induced diabetic erectile dysfunction. *Asian Pac J Reprod* 2017; **6**: 6-12 [DOI: 10.12980/apjr.6.20170102]

87 **Kashif S**, Razdan R, Jamwal R, Goswami SK. Allantoin alleviates male sexual dysfunction in diabetic rats through augmenting the level of testosterone. *J Young Pharm* 2018; **10**: 57-61 [DOI: 10.5530/jyp.2018.10.14]

88 **Culha MG**, Tuken M, Gonultas S, Cakir OO, Serefoglu EC. Frequency of etiological factors among patients with acquired premature ejaculation: prospective, observational, single-center study. *Int J Impot Res* 2020; **32**: 352-357 [PMID: 31477853 DOI: 10.1038/s41443-019-0188-x]

89 **Khan HL**, Bhatti S, Abbas S, Khan YL, Gonzalez RMM, Aslamkhan M, Gonzalez GR, Aydin HH. Longer trinucleotide repeats of androgen receptor are associated with higher testosterone and low oxytocin levels in diabetic premature ejaculatory dysfunction patients. *Basic Clin Androl* 2018; **28**: 3 [PMID: 29556396 DOI: 10.1186/s12610-018-0068-0]

90 **Malavige LS**, Wijesekara P, Seneviratne Epa D, Ranasinghe P, Levy JC. Ethnic differences in sexual dysfunction among diabetic and nondiabetic males: the Oxford Sexual Dysfunction Study. *J Sex Med* 2013; **10**: 500-508 [PMID: 22925588 DOI: 10.1111/j.1743-6109.2012.02911.x]

91 **El-Sakka AI**. Premature ejaculation in non-insulindependent diabetic patients. *Int J Androl* 2003; **26**: 329-334 [DOI: 10.1111/j.1365-2605.2003.00433.x]

92 **Majzoub A**, Arafa M, Al-Said S, Dabbous Z, Aboulsoud S, Khalafalla K, Elbardisi H. Premature ejaculation in type II diabetes mellitus patients: association with glycemic control. *Transl Androl Urol* 2016; **5**: 248-254 [PMID: 27141454 DOI: 10.21037/tau.2016.03.11]

93 **Malavige LS**, Jayaratne SD, Kathriarachchi ST, Sivayogan S, Fernando DJ, Levy JC. Erectile dysfunction among men with diabetes is strongly associated with premature ejaculation and reduced libido. *J Sex Med* 2008; **5**: 2125-2134 [PMID: 18624974 DOI: 10.1111/j.1743-6109.2008.00907.x]

94 **Olamoyegun MA**, Ayodele AO, Yemi FE, Akinyele AT. Prevalence of premature ejaculation among patients with type 2 diabetes in a tertiary health institution. *J Diabetes Mellitus* 2020; **10**: 88-97 [DOI: 10.4236/jdm.2020.102008]

95 **Owiredu WK**, Amidu N, Alidu H, Sarpong C, Gyasi-Sarpong CK. Determinants of sexual dysfunction among clinically diagnosed diabetic patients. *Reprod Biol Endocrinol* 2011; **9**: 70 [PMID: 21612653 DOI: 10.1186/1477-7827-9-70]

96 **Bellastella G**, Maiorino MI, Olita L, Della Volpe E, Giugliano D, Esposito K. Premature ejaculation is associated with glycemic control in Type 1 diabetes. *J Sex Med* 2015; **12**: 93-99 [PMID: 25424355 DOI: 10.1111/jsm.12755]

97 **Corona G**, Giorda CB, Cucinotta D, Guida P, Nada E; Gruppo di studio SUBITO-DE. Sexual dysfunction at the onset of type 2 diabetes: the interplay of depression, hormonal and cardiovascular factors. *J Sex Med* 2014; **11**: 2065-2073 [PMID: 25041930 DOI: 10.1111/jsm.12601]

98 **Dinulovic D**, Radonjic G. Diabetes mellitus/male infertility. *Arch Androl* 1990; **25**: 277-293 [PMID: 2285351 DOI: 10.3109/01485019008987617]

99 **Dunsmuir WD**, Holmes SA. The aetiology and management of erectile, ejaculatory, and fertility

problems in men with diabetes mellitus. *Diabet Med* 1996; **13**: 700-708 [PMID: 8862943 DOI: 10.1002/(SICI)1096-9136(199608)13:8<700::AID-DIA174>3.0.CO;2-8]

100 **Condorelli RA**, Vicari E, Calogero AE, La Vignera S. Male accessory gland inflammation prevalence in type 2 diabetic patients with symptoms possibly reflecting autonomic neuropathy. *Asian J Androl* 2014; **16**: 761-766 [PMID: 24799635 DOI: 10.4103/1008-682X.125911]

101 **La Vignera S**, Condorelli RA, Cannarella R, Giacone F, Mongioi' LM, Cimino L, Defeudis G, Mazzilli R, Calogero AE. Urogenital infections in patients with diabetes mellitus: Beyond the conventional aspects. *Int J Immunopathol Pharmacol* 2019; **33**: 2058738419866582 [PMID: 32031031 DOI: 10.1177/2058738419866582]

102 **Pop-Busui R**, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 136-154 [PMID: 27999003 DOI: 10.2337/dc16-2042]

103 **Culver GJ**, Tannenhaus J. Calcification of the vas deferens in diabetes. *J Am Med Assoc* 1960; **173**: 648-651 [PMID: 13813264 DOI: 10.1001/jama.1960.03020240036006]

104 **Tsuno MM**, Gerber JM, Pokras RS. Calcification of vas deferens associated with diabetes mellitus. *J Manipulative Physiol Ther* 1991; **14**: 70-73 [PMID: 2002291]

105 **Gianatti EJ**, Dupuis P, Hoermann R, Zajac JD, Grossmann M. Effect of testosterone treatment on constitutional and sexual symptoms in men with type 2 diabetes in a randomized, placebo-controlled clinical trial. *J Clin Endocrinol Metab* 2014; **99**: 3821-3828 [PMID: 24978674 DOI: 10.1210/jc.2014-1872]

106 **Morgentaler A**, Polzer P, Althof S, Bolyakov A, Donatucci C, Ni X, Patel AB, Basaria S. Delayed Ejaculation and Associated Complaints: Relationship to Ejaculation Times and Serum Testosterone Levels. *J Sex Med* 2017; **14**: 1116-1124 [PMID: 28807505 DOI: 10.1016/j.jsxm.2017.06.013]

107 **Burke JP**, Jacobson DJ, McGree ME, Nehra A, Roberts RO, Girman CJ, Lieber MM, Jacobsen SJ. Diabetes and sexual dysfunction: results from the Olmsted County study of urinary symptoms and health status among men. *J Urol* 2007; **177**: 1438-1442 [PMID: 17382749 DOI: 10.1016/j.juro.2006.11.059]

108 **Corona G**, Mannucci E, Petrone L, Ricca V, Balercia G, Mansani R, Chiarini V, Giommi R, Forti G, Maggi M. Association of hypogonadism and type II diabetes in men attending an outpatient erectile dysfunction clinic. *Int J Impot Res* 2006; **18**: 190-197 [PMID: 16136189 DOI: 10.1038/sj.ijir.3901391]

109 **Corona G**, Mannucci E, Petrone L, Fisher AD, Balercia G, De Scisciolo G, Pizzocaro A, Giommi R, Chiarini V, Forti G, Maggi M. Psychobiological correlates of delayed ejaculation in male patients with sexual dysfunctions. *J Androl* 2006; **27**: 453-458 [PMID: 16339452 DOI: 10.2164/jandrol.05154]

110 **Klebanow D**, Macleod J. Semen quality and certain disturbances of reproduction in diabetic men. *Fertil Steril* 1960; **11**: 255-261 [PMID: 14409622 DOI: 10.1016/s0015-0282(16)33781-5]

111 **Greene LF**, Kelalis PP. Retrograde ejaculation of semen dueto diabetic neuropathy. *J Urol* 1967; **98**: 696 [PMID: 6080049]

112 **Koyanagi T**. Studies on the sphincteric system located distally in the urethra: the external urethral sphincter revisited. *J Urol* 1980; **124**: 400-406 [PMID: 6107390 DOI: 10.1016/s0022-5347(17)55468-3]

113 **Cao N**, Gu B, Gotoh D, Yoshimura N. Time-Dependent Changes of Urethral Function in Diabetes Mellitus: A Review. *Int Neurourol J* 2019; **23**: 91-99 [PMID: 31260608 DOI: 10.5213/inj.1938050.025]

114 **Basile Fasolo C**, Mirone V, Gentile V, Parazzini F, Ricci E; Andrology Prevention Week centers; Italian Society of Andrology (SIA). Premature ejaculation: prevalence and associated conditions in a sample of 12,558 men attending the andrology prevention week 2001--a study of the Italian Society of Andrology (SIA). *J Sex Med* 2005; **2**: 376-382 [PMID: 16422869 DOI: 10.1111/j.1743-6109.2005.20350.x]

115 **Corona G**, Rastrelli G, Limoncin E, Sforza A, Jannini EA, Maggi M. Interplay Between Premature Ejaculation and Erectile Dysfunction: A Systematic Review and Meta-Analysis. *J Sex Med* 2015; **12**: 2291-2300 [PMID: 26552599 DOI: 10.1111/jsm.13041]

116 **Lee SW**, Lee JH, Sung HH, Park HJ, Park JK, Choi SK, Kam SC. The prevalence of premature ejaculation and its clinical characteristics in Korean men according to different definitions. *Int J Impot Res* 2013; **25**: 12-17 [PMID: 22931761 DOI: 10.1038/ijir.2012.27]

117 **Hoffman RP**. Sympathetic mechanisms of hypoglycemic counterregulation. *Curr Diabetes Rev* 2007; **3**: 185-193 [PMID: 18220670 DOI: 10.2174/157339907781368995]

118 **Xia J**, Zhang Q, Wang Y, Luan J, Yang J, Cong R, Wang Z, Song N. Association of NE, leptin, and 5-HT with electrophysiological parameters in patients with primary premature ejaculation. *Andrology* 2020; **8**: 1070-1075 [PMID: 32068350 DOI: 10.1111/andr.12773]

119 **Serefoglu EC**, McMahon CG, Waldinger MD, Althof SE, Shindel A, Adaikan G, Becher EF, Dean J, Giuliano F, Hellstrom WJ, Giraldi A, Glina S, Incrocci L, Jannini E, McCabe M, Parish S, Rowland D, Segraves RT, Sharlip I, Torres LO. An evidence-based unified definition of lifelong and acquired premature ejaculation: report of the second international society for sexual medicine ad hoc committee for the definition of premature ejaculation. *Sex Med* 2014; **2**: 41-59 [PMID: 25356301 DOI: 10.1002/sm2.27]

120 **Hatzimouratidis K**, Giuliano F, Moncada I, Muneer A, Salonia A, Verze P, Guideline Associates: Parnham A, Serefoglu EC. EAU guidelines on erectile dysfunction, premature ejaculation, penile

curvature and priapism. Arnhem, The Netherlands: EAU Guidelines Office; 2018. Available from: https://www.uroweb.org/guideline/male-sexual-dysfunction

121 **McMahon CG**, Giuliano F, Dean J, Hellstrom WJ, Bull S, Tesfaye F, Sharma O, Rivas DA, Aquilina JW. Efficacy and safety of dapoxetine in men with premature ejaculation and concomitant erectile dysfunction treated with a phosphodiesterase type 5 inhibitor: randomized, placebo-controlled, phase III study. *J Sex Med* 2013; **10**: 2312-2325 [PMID: 23845016 DOI: 10.1111/jsm.12236]

122 **Jiann BP**, Huang YJ. Assessing satisfaction in men with premature ejaculation after dapoxetine treatment in real-world practice. *Int J Clin Pract* 2015; **69**: 1326-1333 [PMID: 26194604 DOI: 10.1111/ijcp.12700]

123 **Tuken M**, Culha MG, Serefoglu EC. Efficacy and safety of dapoxetine/sildenafil combination tablets in the treatment of men with premature ejaculation and concomitant erectile dysfunction-DAP-SPEED Study. *Int J Impot Res* 2019; **31**: 92-96 [PMID: 30705437 DOI: 10.1038/s41443-019-0122-2]

124 **Abdel-Hamid IA**, Ali OI. Delayed Ejaculation: Pathophysiology, Diagnosis, and Treatment. *World J Mens Health* 2018; **36**: 22-40 [PMID: 29299903 DOI: 10.5534/wjmh.17051]

125 **American Psychiatric Association**. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: American Psychiatric Association; 2013

126 **Kaplan HS**. The new sex therapy. London, UK, Baillie Tindall; 1974

127 **Laumann EO**, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. *JAMA* 1999; **281**: 537-544 [PMID: 10022110 DOI: 10.1001/jama.281.6.537]

128 **Di Sante S**, Mollaioli D, Gravina GL, Ciocca G, Limoncin E, Carosa E, Lenzi A, Jannini EA. Epidemiology of delayed ejaculation. *Transl Androl Urol* 2016; **5**: 541-548 [PMID: 27652226 DOI: 10.21037/tau.2016.05.10]

129 **Jangir RN**, Jain GC. Diabetes mellitus induced impairment of male reproductive functions: a review. *Curr Diabetes Rev* 2014; **10**: 147-157 [PMID: 24919656 DOI: 10.2174/1573399810666140606111745]

130 **Salma N**. Sexual dysfunctions as self-reported by diabetic- type-2 men: An Andrology clinic-based study in Alexandria, Egypt. *Am J Med Biol Res* 2013; **1**: 50-57 [DOI: 10.12691/ajmbr-1-3-1]

131 **Gerig NE**, Meacham RB, Ohl DA. Use of electroejaculation in the treatment of ejaculatory failure secondary to diabetes mellitus. *Urology* 1997; **49**: 239-242 [PMID: 9037287 DOI: 10.1016/S0090-4295(96)00444-X]

132 **Fairburn CG**, Wu FC, McCulloch DK, Borsey DQ, Ewing DJ, Clarke BF, Bancroft JH. The clinical features of diabetic impotence: a preliminary study. *Br J Psychiatry* 1982; **140**: 447-452 [PMID: 7104528 DOI: 10.1192/bjp.140.5.447]

133 **Butcher MJ**, Welliver RC Jr, Sadowski D, Botchway A, Köhler TS. How is delayed ejaculation defined and treated in North America? *Andrology* 2015; **3**: 626-631 [PMID: 26013106 DOI: 10.1111/andr.12041]

134 **Abdel-Hamid IA**, Elsaied MA, Mostafa T. The drug treatment of delayed ejaculation. *Transl Androl Urol* 2016; **5**: 576-591 [PMID: 27652229 DOI: 10.21037/tau.2016.05.05]

135 **Agochukwu-Mmonu N**, Pop-Busui R, Wessells H, Sarma AV. Autonomic neuropathy and urologic complications in diabetes. *Auton Neurosci* 2020; **229**: 102736 [PMID: 33197694 DOI: 10.1016/j.autneu.2020.102736]

136 **Lu S**, Cui Y, Li X, Zhang H, Hu J, Liu J, Chen ZJ. Sperm retrieval in anejaculatory diabetic men who failed in drug treatment and penile vibratory stimulation during blood sugar under control. *Andrologia* 2014; **46**: 370-373 [PMID: 23488950 DOI: 10.1111/and.12087]

137 **Castle SM**, Jenkins LC, Ibrahim E, Aballa TC, Lynne CM, Brackett NL. Safety and efficacy of a new device for inducing ejaculation in men with spinal cord injuries. *Spinal Cord* 2014; **52** Suppl 2: S27-S29 [PMID: 25082380 DOI: 10.1038/sc.2014.110]

138 **Lucas MG**, Hargreave TB, Edmond P, Creasey GH, McParland M, Seager SW. Sperm retrieval by electro-ejaculation. Preliminary experience in patients with secondary anejaculation. *Br J Urol* 1991; **67**: 191-194 [PMID: 2004235 DOI: 10.1111/j.1464-410x.1991.tb15108.x]

139 **Hovatta O**, Reima I, Foudila T, Butzow T, Johansson K, von Smitten K. Vas deferens aspiration and intracytoplasmic injection of frozen-thawed spermatozoa in a case of anejaculation in a diabetic man. *Hum Reprod* 1996; **11**: 334-335 [PMID: 8671220 DOI: 10.1093/humrep/11.2.334]

140 **Fang J**, Shu L, Cai L, Cui Y, Liu J, Yang X. Intracytoplasmic sperm injection outcomes in patients with orgasmic dysfunction and anejaculation by percutaneous epididymal sperm aspiration (PESA). *Ann Transl Med* 2020; **8**: 1214 [PMID: 33178746 DOI: 10.21037/atm-20-1121a]

141 **Alrabeeah K**, Yafi F, Flageole C, Phillips S, Wachter A, Bissonnette F, Kadoch IJ, Zini A. Testicular sperm aspiration for nonazoospermic men: sperm retrieval and intracytoplasmic sperm injection outcomes. *Urology* 2014; **84**: 1342-1346 [PMID: 25432825 DOI: 10.1016/j.urology.2014.08.032]

142 **Jefferys A**, Siassakos D, Wardle P. The management of retrograde ejaculation: a systematic review and update. *Fertil Steril* 2012; **97**: 306-312 [PMID: 22177462 DOI: 10.1016/j.fertnstert.2011.11.019]

143 **Kam J**, Tsang VH, Chalasani V. Retrograde Ejaculation: A Rare Presenting Symptom of Type 1 Diabetes Mellitus. *Urol Case Rep* 2017; **10**: 9-10 [PMID: 27818945 DOI: 10.1016/j.eucr.2016.10.008]

144 **Takahashi Y**, Okawa S. Investigation of sexual life in type I diabetic patients. *Jpn J Impot Res*

2000; **15**: 263-264

145 **Kurbatov D**, Russo GI, Galstyan GR, Rozhivanov R, Lepetukhin A, Dubsky S, Shwartz YG, Cimino S, Morgia G, Sansalone S. Correction of Retrograde Ejaculation in Patients with Diabetes Mellitus Using Endourethral Collagen Injection: Preliminary Results. *J Sex Med* 2015; **12**: 2126-2129 [PMID: 26553413 DOI: 10.1111/jsm.13024]

146 **Otani T**. Clinical review of ejaculatory dysfunction. *Reprod Med Biol* 2019; **18**: 331-343 [PMID: 31607793 DOI: 10.1002/rmb2.12289]

147 **Ibragimov AZ**, Aliev TA, Abdullaev KI, Mirza-Zade VA. Sostoianie zamykatel'nogo apparata mochevogo puzyria pri retrogradnoĭ éiakuliatsii u bol'nykh sakharnym diabetom [The function of the closure apparatus of the bladder in retrograde ejaculation in diabetics]. *Urol Nefrol (Mosk)* 1990; 65-68 [PMID: 2396344]

148 **Mazzilli R**, Defeudis G, Olana S, Zamponi V, Macera M, Mazzilli F. The role of ejaculatory dysfunction on male infertility. *Clin Ter* 2020; **171**: e523-e527 [PMID: 33151251 DOI: 10.7417/CT.2020.2267]

149 **McMahon CG**. Disorders of Male Orgasm and Ejaculation. In: Wein AJ, Kavoussi LR, Partin AW. Campbell-Walsh Urology. Philadelphia: Elsevier, 2016: 692-708

150 **Fedder J**, Kaspersen MD, Brandslund I, Højgaard A. Retrograde ejaculation and sexual dysfunction in men with diabetes mellitus: a prospective, controlled study. *Andrology* 2013; **1**: 602-606 [PMID: 23606485 DOI: 10.1111/j.2047-2927.2013.00083.x]

151 **McMahon CG**, Abdo C, Incrocci L, Perelman M, Rowland D, Waldinger M, Xin ZC. Disorders of orgasm and ejaculation in men. *J Sex Med* 2004; **1**: 58-65 [PMID: 16422984 DOI: 10.1111/j.1743-6109.2004.10109.x]

152 **Andaloro VA Jr**, Dube A. Treatment of retrograde ejaculation with brompheniramine. *Urology* 1975; **5**: 520-522 [PMID: 1129870 DOI: 10.1016/0090-4295(75)90080-1]

153 **Budd HA Jr**. Letter: Brompheniramine in treatment of retrograde ejaculation. *Urology* 1975; **6**: 131 [PMID: 1145917 DOI: 10.1016/0090-4295(75)90614-7]

154 **Stewart BH**, Bergant JA. Correction of retrograde ejaculation by sympathomimetic medication: preliminary report. *Fertil Steril* 1974; **25**: 1073-1074 [PMID: 4430388 DOI: 10.1016/s0015-0282(16)40816-2]

155 **Gilja I**, Parazajder J, Radej M, Cvitković P, Kovacić M. Retrograde ejaculation and loss of emission: possibilities of conservative treatment. *Eur Urol* 1994; **25**: 226-228 [PMID: 8200405 DOI: 10.1159/000475288]

156 **Arafa M**, El Tabie O. Medical treatment of retrograde ejaculation in diabetic patients: a hope for spontaneous pregnancy. *J Sex Med* 2008; **5**: 194-198 [PMID: 17433085 DOI: 10.1111/j.1743-6109.2007.00456.x]

157 **Shoshany O**, Abhyankar N, Elyaguov J, Niederberger C. Efficacy of treatment with pseudoephedrine in men with retrograde ejaculation. *Andrology* 2017; **5**: 744-748 [PMID: 28380686 DOI: 10.1111/andr.12361]

158 **Brooks ME**, Berezin M, Braf Z. Treatment of retrograde ejaculation with imipramine. *Urology* 1980; **15**: 353-355 [PMID: 7190335 DOI: 10.1016/0090-4295(80)90468-9]

159 **Okada H**, Goda K, Koshida M, Kamidono S. Pregnancy by insemination of cryopreserved spermatozoa from a man with retrograde ejaculation: a case report. *J Reprod Med* 2004; **49**: 389-391 [PMID: 15214715]

160 **Eppel SM**, Berzin M. Pregnancy following treatment of retrograde ejaculation with clomipramine hydrochloride. A report of 3 cases. *S Afr Med J* 1984; **66**: 889-891 [PMID: 6505897]

161 **Hibi H**, Ohori T, Yamada Y. DPP-IV inhibitor may affect spermatogenesis. *Diabetes Res Clin Pract* 2011; **93**: e74-e75 [PMID: 21636156 DOI: 10.1016/j.diabres.2011.04.022]

162 **Templeton A**, Mortimer D. Successful circumvention of retrograde ejaculation in an infertile diabetic man. Case report. *Br J Obstet Gynaecol* 1982; **89**: 1064-1065 [PMID: 7171519 DOI: 10.1111/j.1471-0528.1982.tb04668.x]

163 **Brassesco M**, Viscasillas P, Burrel L, Calaf J, Rajmil O, Pomerol Serra JM, Morer Fargas F. Sperm recuperation and cervical insemination in retrograde ejaculation. *Fertil Steril* 1988; **49**: 923-925 [PMID: 3360184 DOI: 10.1016/s0015-0282(16)59909-9]

164 **Shangold GA**, Cantor B, Schreiber JR. Treatment of infertility due to retrograde ejaculation: a simple, cost-effective method. *Fertil Steril* 1990; **54**: 175-177 [PMID: 2358086 DOI: 10.1016/s0015-0282(16)53660-7]

165 **Hotchkiss RS**, Pinto AB, Kleegman S. Artificial insemination with semen recovered from the bladder. *Fertil Steril* 1954; **6**: 37-42 [PMID: 13220644 DOI: 10.1016/s0015-0282(16)31863-5]

166 **Palermo GD**, O'Neill CL, Chow S, Cheung S, Parrella A, Pereira N, Rosenwaks Z. Intracytoplasmic sperm injection: state of the art in humans. *Reproduction* 2017; **154**: F93-F110 [PMID: 29158352 DOI: 10.1530/REP-17-0374]

167 **Philippon M**, Karsenty G, Bernuz B, Courbiere B, Brue T, Saïas-Magnan J, Perrin J. Successful pregnancies and healthy live births using frozen-thawed sperm retrieved by a new modified Hotchkiss procedure in males with retrograde ejaculation: first case series. *Basic Clin Androl* 2015; **25**: 5 [PMID: 26034605 DOI: 10.1186/s12610-015-0021-4]

168 **Liu X**, Gao M, Sun J, Sun Z, Song J, Xue X, Zhang Z, Shi J, Xing J. Effects of testicular sperm aspiration upon first cycle ICSI-ET for type 2 diabetic male patients. *Syst Biol Reprod Med* 2020; **66**: 355-363 [PMID: 32717167 DOI: 10.1080/19396368.2020.1785042]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 975-996

DOI: 10.4239/wjd.v12.i7.975

ISSN 1948-9358 (online)

REVIEW

Diabetic patients with chronic kidney disease: Non-invasive assessment of cardiovascular risk

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Author contributions: Piko N, Bevc S and Hojs R contributed to the conception and design of the manuscript; Piko N, Bevc S, Hojs R, Petreski T, Vodošek Hojs N and Ekart R drafted the article, made critical revisions related to the manuscript and approved the final version of the manuscript to be published.

Conflict-of-interest statement: The authors declare no conflict of interest exists. This article has previously not been published elsewhere in any shape or form.

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Abstract

The prevalence and burden of diabetes mellitus and chronic kidney disease on global health and socioeconomic development is already heavy and still rising. Diabetes mellitus by itself is linked to adverse cardiovascular events, and the presence of concomitant chronic kidney disease further amplifies cardiovascular risk. The culmination of traditional (male gender, smoking, advanced age, obesity, arterial hypertension and dyslipidemia) and non-traditional risk factors (anemia, inflammation, proteinuria, volume overload, mineral metabolism abnormalities, oxidative stress, *etc.*) contributes to advanced atherosclerosis and increased cardiovascular risk. To decrease the morbidity and mortality of these patients due to cardiovascular causes, timely and efficient cardiovascular risk assessment is of huge importance. Cardiovascular risk assessment can be based on laboratory parameters, imaging techniques, arterial stiffness parameters, ankle-brachial index and 24 h blood pressure measurements. Newer methods include epigenetic markers, soluble adhesion molecules, cytokines and markers of oxidative stress. In this review, the authors present several non-invasive methods of cardiovascular risk assessment in patients with diabetes mellitus and chronic kidney disease.

Key Words: Diabetes mellitus; Diabetes complications; Chronic kidney disease; Atherogenesis; Atherosclerosis; Cardiovascular risk

Manuscript source: Invited manuscript

Specialty type: Cardiac and cardiovascular systems

Country/Territory of origin: Slovenia

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 26, 2021
Peer-review started: January 26, 2021
First decision: February 25, 2021
Revised: March 4, 2021
Accepted: April 26, 2021
Article in press: April 26, 2021
Published online: July 15, 2021

P-Reviewer: Kwon SK, Ong H
S-Editor: Zhang L
L-Editor: Filipodia
P-Editor: Li JH

Core Tip: The culmination of traditional and non-traditional atherosclerosis risk factors in patients with diabetes mellitus and chronic kidney disease leads to fulminant and advanced atherosclerosis, consequently resulting in cardiovascular morbidity and mortality. Non-invasive cardiovascular risk assessment should therefore be performed in all these patients and can be based on standard laboratory parameters, cytokines and markers of oxidative stress, 24 h blood pressure measurements, ankle-brachial index, arterial stiffness parameters, imaging techniques and epigenetic markers. In this review article, we present different methods of non-invasive cardiovascular risk assessment in patients with diabetes mellitus and chronic kidney disease.

Citation: Piko N, Bevc S, Ekart R, Petreski T, Vodošek Hojs N, Hojs R. Diabetic patients with chronic kidney disease: Non-invasive assessment of cardiovascular risk. *World J Diabetes* 2021; 12(7): 975-996
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/975.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.975

INTRODUCTION

The burden of diabetes mellitus (DM) on global public health and socioeconomic development is heavy and is escalating. The International Diabetes Foundation has stated that in 2017, 451 million people worldwide have lived with DM and that this number would soar in the next couple of years and decades[1].

DM is the number one cause of chronic kidney disease (CKD) worldwide and is inherently linked with increased mortality mostly due to cardiovascular causes[2]. CKD is another major public concern with rising prevalence, which is now estimated at 9.1%[3]. In 2017, CKD resulted in 1.2 million deaths and was the 12th leading cause of death globally. Additionally, 7.6% of all cardiovascular deaths were due to CKD[4].

Cardiovascular disease in patients with DM and CKD is attributed to advanced and fulminant atherosclerosis, due to the presence and interplay of traditional [male gender, smoking, advanced age, obesity, arterial hypertension (AH), dyslipidemia] and non-traditional, CKD-specific risk factors (anemia, inflammation, proteinuria, volume overload, mineral metabolism abnormalities, oxidative stress, *etc.*)[5].

Due to the increased rate of cardiovascular events, prompt and timely cardiovascular risk assessment is crucial in reducing the morbidity and mortality of these patients[6]. In this review article, the authors present non-invasive methods of assessing cardiovascular risk in patients with DM and CKD (Figure 1).

STANDARD LABORATORY PARAMETERS

Estimated glomerular filtration rate

The diagnosis of CKD is based on functional and structural changes of the kidneys, with the former assessed by estimating or measuring the glomerular filtration rate (GFR) and albuminuria/proteinuria and the latter by using different imaging techniques and/or kidney biopsy. Multiple creatinine and/or cystatin C based equations are only an assessment of kidney function and have several limitations, but the alternative (measurements of GFR) is usually not available in routine clinical practice[7].

Reduced estimated GFR is an important marker of cardiovascular risk. Go *et al*[8] performed a study in which they estimated the longitudinal GFR among 1120295 adults in whom serum creatinine had been measured between 1996-2000 and who had not undergone dialysis or kidney transplantation. Of the included patients, 9.6% had concomitant DM. They found an independent, graded increase in cardiovascular deaths, events and hospitalization rates in those with reduced estimated GFR. The authors postulated that the relationship between reduced GFR and cardiovascular disease is partly due to the presence of several traditional atherosclerosis risk factors and partly due to the presence of several CKD-specific risk factors, such as increased levels of inflammation markers, hyperhomocysteinemia, abnormal apolipoprotein levels, enhanced coagulability, anemia, left ventricular hypertrophy (LVH), increased

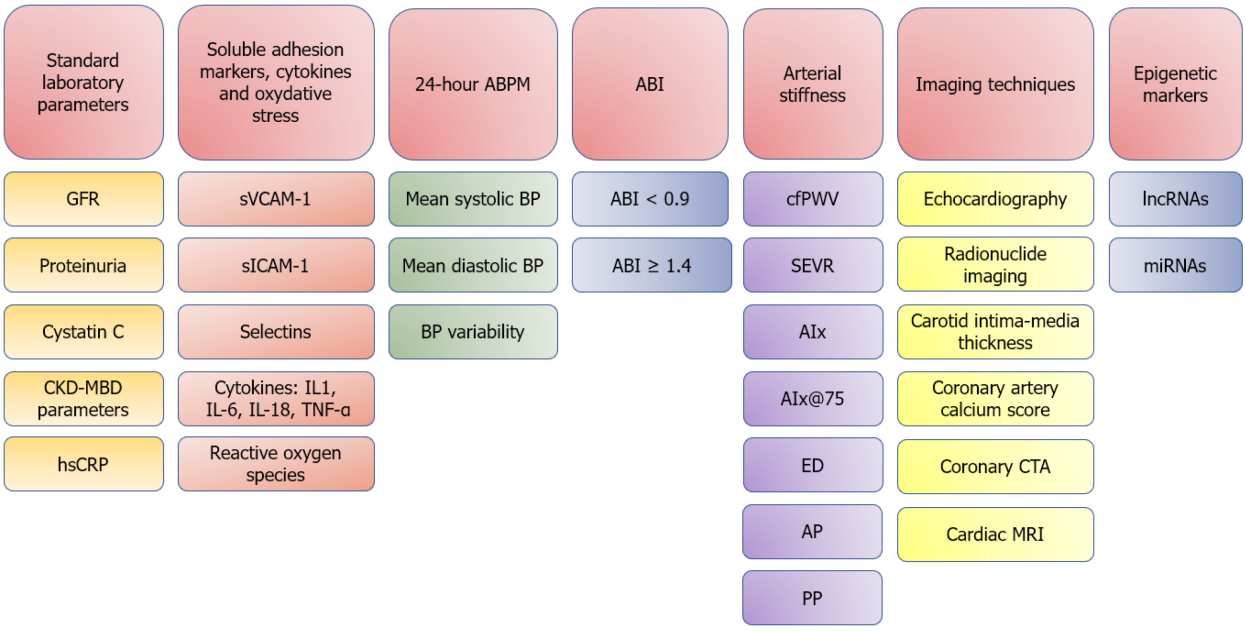

Figure 1 Non-invasive assessment of cardiovascular risk in patients with diabetes mellitus and chronic kidney disease. GFR: Glomerular filtration rate; CKD-MBD: Chronic kidney disease mineral bone disorder; hsCRP: High sensitivity C-reactive protein; sVCAM-1: Soluble vascular cell adhesion molecule-1; sICAM-1: Soluble intercellular adhesion molecule-1; IL-1:Interleukin-1; IL-6: Interleukin-6; IL-18: Interleukin-18; TNF-α: Tumor necrosis factor-α; ABPM: Ambulatory blood pressure measurements; BP: Blood pressure; ABI: Ankle-brachial index; cfPWV: Carotid-femoral pulse wave velocity; SEVR: Subendocardial viability ratio; AIx: Augmentation index; AIx@75: AIx adjusted for heart rate 75/min; ED: Ejection duration; AP: Augmentation pressure; PP: Pulse pressure; CTA: Computed tomography angiography; MRI: Magnetic resonance imaging; lncRNAs: Long non-coding ribonucleic acids; miRNAs: Micro-ribonucleic acids.

endothelial dysfunction, increased arterial stiffness and augmented arterial calcifications.

In a study by Wu *et al*[9], the authors found that reduced estimated GFR was an important hallmark of diabetic retinopathy, which is commonly associated with advanced diabetic nephropathy and increased cardiovascular risk as well. In a 1-year cross-sectional study by Babaliche *et al*[10], the authors found that reduced GFR in type 2 DM patients was significantly associated with a higher incidence of microvascular complications, such as diabetic neuropathy, retinopathy and nephropathy.

DM exerts its negative effects on kidney function through negative glomerular hemodynamic effects, especially through glomerular hyperfiltration, which is defined as an estimated GFR more than two standard deviations above the mean estimated GFR of healthy individuals. In a study performed on healthy, middle-aged individuals by Dupuis *et al*[11], the authors found that glomerular hyperfiltration is independently associated with higher cardiovascular risk, similar to the risk observed in patients with CKD stage 3A. Similar findings have been observed in several studies on patients with type 1 and type 2 DM, with and without previous cardiovascular disease[12-14]. Glomerular hyperfiltration was also associated with an increased incidence of coronary artery calcification and LVH, both important cardiovascular risk markers[15].

Proteinuria

Proteinuria (defined as urine protein excretion greater than 300 mg over 24 h) and moderately increased albuminuria (defined as urinary albumin excretion of 30-300 mg over 24 h) are usually signs of renal injury and can often be detected earlier than any tangible fall in GFR[16]. Both are strong and independent predictors of increased risk for all-cause and cardiovascular mortality in patients with and without DM. Additionally, both are risk factors for faster CKD progression as well and are therefore included in the KDIGO (kidney disease: Improving global outcomes) CKD staging system[16].

In a post-hoc analysis of the Reduction in Endpoint in Non-insulin dependent DM with the angiotensin II antagonist losartan trial, the authors found that proteinuria of 3 g or more per day was associated with a renal endpoint of doubling of creatinine or end-stage renal disease (ESRD) in 85% of patients and with a cardiovascular endpoint (defined as the composite of myocardial infarction, stroke, first hospitalization for heart failure or unstable angina, coronary or peripheral revascularization or cardiovascular death) in 44% of patients. In the same study, moderately increased

albuminuria was associated with an increased likelihood of kidney disease progression and cardiovascular risk as well, even after adjusting for estimated GFR and comorbidities[17].

The Strong Heart Study showed that patients with type 2 DM and proteinuria had worse left ventricular function and impaired diastolic left ventricular filling compared with patients without proteinuria[18]. According to studies, diabetic patients with proteinuria are at increased risk for peripheral artery disease (PAD)[19] and incident stroke as well[20].

In a Heart Outcome Prevention Evaluation (HOPE) substudy, a linear relationship between moderately increased albuminuria and cardiovascular events, especially in patients aged 55 years or more with previous cardiovascular disease or DM, was proven. In the same study, a higher incidence of systolic and diastolic dysfunction was found in patients with moderately increased albuminuria[21]. A study of 308 patients showed that patients with angiographic evidence of coronary artery disease (CAD) had higher urinary albumin levels than disease-free individuals and that urinary albumin excretion increased progressively with CAD severity[22].

All the above-stated studies confirm the fact that the detection and early recognition of moderately increased albuminuria and proteinuria is crucial in managing cardiovascular risk and the rate of CKD progression[23,24].

The exact pathophysiologic pathways that would explain the mechanism behind increased cardiovascular risk and proteinuria are still not known. It is postulated that the proteins that leak through damaged glomerular capillary endothelium cause tubulointerstitial injury and inflammation and subsequently lead to parenchymal damage, renal fibrosis and progressive decline in renal function[25]. The steno hypothesis suggests that urinary protein excretion signals a subclinical renal disease and systemic endothelial dysfunction and systemic inflammation[26]. Additionally, several thrombogenic factors, for example, von Willebrand factor, fibrinogen, cell adhesion molecules and tissue plasminogen activator have also been connected with proteinuria. It has been suggested that high platelet adhesiveness and erythrocyte aggregation demonstrated in diabetic patients with proteinuria could indicate increased thrombosis risk. Insulin resistance has been proven in patients with proteinuria, implying the role of hyperinsulinism in explaining the increased cardiovascular risk in these patients[26].

Cystatin C

Serum cystatin C is a low-molecular-weight, non-glycosylated protein from the family of cysteine protease inhibitors that closely approximates what could be considered an ideal marker of renal function because it is freely filtered by the glomerulus, reabsorbed and degraded completely by proximal tubule and is not secreted by the tubules. It is more sensitive than usual endogenous markers (serum creatinine and urea) used in kidney function assessment, especially in the early stages of CKD[27]. However, cystatin C can also be used as a cardiovascular risk marker having important prognostic implications in patients with different degrees of CKD[28].

According to a cross-sectional epidemiological study by Cepeda *et al*[29], elevated cystatin C was associated with an increased presence of several cardiovascular risk factors, such as DM, AH and CKD, along with higher levels of C-reactive protein (CRP), homocysteine and fibrinogen. Correa *et al*[30] performed a study on 4965 individuals after acute coronary syndrome in which they found a higher likelihood of adverse cardiovascular events in those with increased cystatin C, opening up a potential new role of cystatin C in risk stratification. Madero *et al*[31] performed an arterial stiffness study on 2468 individuals (24% diabetic) and found an association between cystatin C and increased arterial stiffness, especially in older patients. In two mortality studies, elevated cystatin C levels were associated with increased all-cause mortality, even in patients with normal renal function[32,33].

It has been suggested that cystatin C exerts its function through inhibition of lysosomal cathepsins, which leads to a reduction in atherosclerotic plaque degradation and increased risk of cardiovascular events[34].

Calcium, phosphate, parathyroid hormone, fibroblast growth factor-23 and vitamin D homeostasis

CKD mineral and bone disorder (CKD-MBD) refers to the clinical syndrome of laboratory abnormalities, bone disease and extraskeletal calcification, including the arterial system. Among the earliest manifestations of CKD-MBD are vitamin D deficiency, disordered calcium and phosphate homeostasis, elevated levels of parathyroid hormone and fibroblast growth factor-23 (FGF-23). These alterations lead

to an increased risk of ESRD, cardiovascular disease and mortality[35]. The amount of evidence is especially strong for elevated serum phosphate and FGF-23, both of which have direct negative cardiovascular effects through promoting LVH and consequent left ventricular dysfunction[36,37]. Several cross-sectional studies have demonstrated that vitamin D deficiency also increases the risk of developing AH, heart failure and sudden cardiac death, all through downregulation of the renin-angiotensin-aldosterone system, impaired insulin sensitivity, direct effects on the heart and vasculature and through worsened glycemic control[38,39].

The difference in CKD-MBD according to diabetes status has also been noticed. In a large descriptive study of patients with CKD stages 2-4, participants with DM had higher levels of serum phosphate, parathyroid hormone and FGF-23 and lower levels of vitamin D compared to patients without DM. Moreover, the elements of CKD-MBD evolved earlier in the course of CKD in diabetic patients, partly explaining the higher cardiovascular risk in diabetic CKD patients. An inverse relationship between the level of proteinuria and vitamin D was also observed, further confirming the importance of surveillance of mineral metabolism in diabetic CKD patients[40].

High-sensitivity CRP

Chronic, low-grade inflammation plays a grand role in the initiation and progression of atherothrombosis, metabolic disorders, AH, DM and renal disease[41]. High-sensitivity CRP (hsCRP) is an established inflammatory biomarker and is one of the most widely used markers associated with risk of cardiovascular events[42]. In the study by Ridker *et al*[43], 27939 presumed healthy American women were followed up for a mean of 8 years for incident myocardial infarction, ischemic stroke, coronary revascularization or death from cardiovascular causes. The authors found that cardiovascular events were more common in those with higher hsCRP levels, independent of traditional atherosclerosis risk factors. In the Cardiovascular Health Study, nearly 6000 subjects were followed up for 3-4 years, and their inflammatory markers were measured before and after completed follow-up. The results of the study showed that those with higher baseline hsCRP values were more likely to develop DM in the follow-up period[44]. An association has also been found between higher values of glycated hemoglobin and hsCRP, suggesting the role of systemic inflammation in diminished insulin sensitivity and suboptimal glycemic control[45].

Interestingly, studies have not shown uniform results regarding the role of hsCRP as a marker of cardiovascular risk in diabetic patients. In a pooled analysis of 25797 patients from four different United Kingdom prospective cohort studies, hsCRP was linked to increased risk of cardiovascular events and mortality only in patients without DM[46]. Similar results were found in the Jager *et al*[47] study and in the Strong Heart Study as well[48]. In the Diabetes Heart Study, hsCRP was analyzed in 846 type 2 DM patients who had follow-ups for a mean period of 7.3 years. On the contrary to other studies, baseline hsCRP was a strong predictor of mortality in this group of patients[49].

Elevated hsCRP has been found to be an important marker of acute kidney injury, subclinical kidney injury, incident CKD and CKD progression[50,51]. Sinha *et al*[52] found that higher baseline hsCRP is associated with incident diabetic nephropathy. In a study by Jalal *et al*[53] in which 3166 elderly subjects were included (18% diabetics), high hsCRP was associated with a higher likelihood of major adverse cardiovascular events. In a meta-analysis by Li *et al*[54] on CKD and ESRD patients, hsCRP was associated with a significantly higher risk of cardiovascular morbidity and mortality. Besides being an important biomarker of cardiovascular risk, a change in hsCRP can be a sign of changed renal and consequently cardiovascular risk profile. Liu *et al*[55] found that a reduction in hsCRP favored kidney outcomes in patients with impaired glucose metabolism or DM, showing that serial measurements of hsCRP can be indicative of change in systemic inflammation and atherogenesis.

SOLUBLE ADHESION MOLECULES, CYTOKINES AND OXIDATIVE STRESS

Inflammation underlies all stages of atherosclerotic plaque formation, even in the early stages where inflammatory cells adhere and infiltrate the subendothelium. The two most important adhesion molecules that play a major role in atherosclerosis are vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Selectins are another group of adhesion molecules and are found on leukocytes, endothelial cells and thrombocytes[56]. Cell adhesion molecules and

selectins can be broken off into the circulation and can be measured in the serum. Soluble adhesion molecules have shown potential as biomarkers of cardiovascular risk [57].

Increased levels of soluble VCAM-1 (sVCAM-1) and soluble ICAM-1 (sICAM-1) have been found in diabetics with increased stages of CKD[57,58]. In a study by Bavbek *et al*[56], increased serum levels of selectins were associated with microvascular complications of DM, including proteinuria and decreased estimated GFR. Becker *et al*[59] demonstrated increased 10-year cardiovascular mortality in diabetic patients with increased sICAM-1 levels, independent of other known traditional cardiovascular risks. Both sICAM-1 and sVCAM-1 were higher in elderly patients with type 2 DM with cerebrovascular and cardiovascular disease[60,61].

Another important component of the inflammatory process in atherosclerosis are cytokines and their receptors. Interleukin (IL)-2 receptor has been associated with increased carotid intima-media thickness (IMT) and advanced atherosclerosis in hemodialysis patients[62]. Population-based studies have shown that inflammatory cytokines are strong predictors of the development of DM and are also important in the development of micro- and macrovascular complications in diabetic patients[63]. IL-1, IL-6, IL-18 and tumor necrosis factor-alpha enhance the expression of ICAM-1, VCAM-1 and selectins, affect the dynamics of the mesangial matrix, lead to glomerular basal membrane thickening, alter glomerular hemodynamics and lead to renal toxicity [64,65]. Cytokines can also lead to dysregulation between antioxidative mechanisms and the formation of reactive oxygen species, such as superoxide anions, hydrogen peroxide and hydroxyl radicals. Ineffective antioxidant capacity or excess reactive oxygen species is implicated in the development and progression of renal and cardiovascular disease through several pathophysiologic mechanisms, including through direct tissue toxicity and promotion of further inflammation[66-68].

TWENTY-FOUR HOUR AMBULATORY BLOOD PRESSURE MEASUREMENTS

In patients with AH, multiple blood pressure measurements during longer periods of time [for example 24 h ambulatory blood pressure measurements (ABPM)] are more likely to predict cardiovascular risk and are therefore superior to those obtained in the doctor's office for estimating the risk of future cardiovascular morbidity and mortality [69].

In a population-based prospective study on 1700 Danish people by Hansen *et al*[70], the authors found that 24 h ABPM was an important prognostic tool in assessing subjects' cardiovascular risks and that isolated ambulatory systolic hypertension as well as blunted decrease in blood pressure during the night were both prognostically unfavorable. The percentage of DM was higher in the sustained high blood pressure group (4.3%) compared to the isolated ambulatory hypertension group (2.8%), isolated office hypertension group (1.3%) and normotensive group (1.2%).

In a cohort study by Grezzana *et al*[71] that included 569 hypertensive patients, the 24 h ABPM showed a predictive result for new cases of atrial fibrillation and a combination of cardiovascular outcomes, mortality and hospital admissions. In a study by Iqbal *et al*[72] in which 1187 individuals were included, significant associations were found between cerebrovascular events and absent nocturnal drop in blood pressure (≤ 10%), between high day time diastolic blood pressure, PAD and morning surge ≥ 20/15 mmHg and between cardiac arrhythmias, high day time and nighttime diastolic blood pressure.

In patients with and without DM, 24 h ABPM is superior to office recordings in terms of recognizing masked and white coat hypertension[73]. A lack of nocturnal dip in blood pressure is suggestive of autonomic neuropathy and is commonly observed in diabetic patients and can very commonly be a sign of concomitant obstructive sleep apnea, which is a known cardiovascular risk factor[74]. In a study by Eguchi[75] performed on patients with type 2 DM, 24 h systolic blood pressure was significantly correlated with silent cerebral infarcts and LVH, even more so than the values of glycosylated hemoglobin, indicating that perhaps uncontrolled hypertension is the main cause of accelerated atherosclerosis and increased cardiovascular risk in this population.

Like DM, CKD is also associated with a distinctive blood pressure profile, resulting in undiagnosed hypertension, which is a major factor in a continuing decline in kidney function. Manios *et al*[76] demonstrated that short-term blood pressure variability is more pronounced in CKD patients, rendering office measurements obsolete and

imprecise in this population. Similar studies have confirmed these findings and additionally showed a larger presence of non-dippers and patients with masked hypertension in the CKD group as well[77]. Decreased diurnal blood pressure variation is independently associated with a faster decline in kidney function[78] and with increased cardiovascular mortality in CKD patients[79]. In the prospective African American study of kidney disease and hypertension cohort study of 617 CKD patients, Gabbai *et al*[80] demonstrated that 24 h systolic blood pressure predicted both kidney and cardiovascular outcomes. Wang *et al*[81] studied a large (n = 1219) cohort of diabetic and non-diabetic CKD patients and found that blood pressure load and ABPM levels were independently correlated with left ventricle mass index, estimated GFR and proteinuria in all groups of CKD patients. Besides CKD stages 2-4, studies have shown an adverse cardiovascular profile in ESRD patients with elevated 24 h systolic blood pressure and a non-dipping ABPM profile as well[82-84].

ANKLE-BRACHIAL INDEX

PAD is usually diagnosed by ankle-brachial index (ABI) < 0.9 and can be considered a clinical model for atherosclerosis. It is the result of structural and functional vessel wall aberrations, resulting in limb ischemia and changes in pulse wave propagation, ultimately impacting the myocardium as well[85]. PAD is an independent risk factor for stroke, myocardial infarction and cardiovascular death, and the risk is even more apparent in diabetic patients[86]. In a study by Li *et al*[87] that was performed on 1647 patients with type 2 DM, low ABI (≤ 0.9) was independently associated with a high risk of all-cause and cardiovascular mortality. In a retrospective study by Alves-Cabratosa *et al*[88] in which 34689 patients with type 2 DM were included, the authors found that even ABI in the lower normal range (0.91-1.00) was associated with significantly increased risk for nephropathy, retinopathy, acute myocardial infarction and mortality. In the same study, high ABI (> 1.3) was a marker of increased medial calcinosis and was associated with cardiovascular complications, most likely due to increased arterial stiffness, directly leading to increased cardiac workload, left ventricular hypertrophy, myocardial fibrosis, ischemia and arrhythmias[89].

CKD patients are especially prone to complications of atherosclerosis, PAD, calcifications of arterial walls and increased arterial stiffness[90]. According to the Cardiovascular Health Study, in which 4.513 community-living subjects aged 65 years or more were enrolled (15.3% diabetics), CKD was associated with both high (> 1.4) and low (< 0.9) extremes of ABI, which was explained by advanced atherosclerosis and increased vessel wall calcifications in this subgroup[90]. The prevalence of medial calcinosis has been shown to be even higher in patients on hemodialysis[91]. The results of the prospective NEFRONA study, in which 2445 CKD and 559 non-CKD subjects were included, showed higher prevalence of PAD in CKD patients and a rising prevalence of ABI > 1.4 in advanced stages of CKD. DM was the only factor predicting both pathological values of ABI in all CKD stages[92].

Both high and low ABI measurements play a role in assessing cardiovascular risk and renal outcome. According to a study by Chen *et al*[93] in which 436 CKD patients were enrolled (36.9% with DM type 2), reduced ABI (< 0.9) was associated with a more rapid decline in renal function and with a higher incidence of cardiovascular events. Similar association was found between renal function, cardiovascular events and high ABI[94]. In a study on 52 hemodialysis patients by Bevc *et al*[95], survival analysis showed higher risk for cardiovascular death in patients with ABI > 1.4. It appears that the systemic nature of atherosclerosis is only partly responsible for these effects of changed ABI and that increased arterial stiffness and consequent hemodynamic changes play an integral role as well[96].

ARTERIAL STIFFNESS PARAMETERS

Increased arterial stiffness is recognized as a surrogate endpoint for cardiovascular disease and is the result of several structural alterations in the arterial walls, leading to reduced distensibility and decreased buffering capacity of arteries to pulsatile cardiac ejection[97].

Applanation tonometry is a non-invasive, easily reproducible technique often used for measuring arterial stiffness. It enables us to perform pulse wave analysis on the radial artery, giving us information on indirect parameters of central arterial stiffness and blood supply to the endocardium of the heart[98]. It also allows us to measure

carotid-femoral pulse wave velocity (cfPWV) on the carotid and femoral arteries, which is the most precise way to non-invasively determine central arterial stiffness [99]. All the applanation tonometry-derived arterial stiffness parameters are presented in Table 1.

Increased arterial stiffness is a well-known risk factor for major cardiovascular events. Weber *et al*[100] performed a prospective study on 465 male patients undergoing coronary angiography and found that augmentation pressure, augmentation index (AIx), and AIx@75 (AIx adjusted for heart rate 75/min) were strong predictors of obstructive CAD. Prskalo *et al*[101] performed a study on 160 patients with CAD undergoing elective coronary angiography and found that increased values of PWV and AIx were associated with a more advanced CAD, a higher likelihood of in-stent restenosis and left main CAD. An association between PAD and increased arterial stiffness has also been described[96]. A prospective Rotterdam study involving 2835 volunteers aged 55 years or more reported that subjects with higher values of cfPWV had a higher risk of coronary and cerebrovascular events[102]. A population Hoorn study included 261 healthy subjects and 358 patients with prediabetes or diabetes and found that the latter group had significantly higher values of cfPWV and AIx, indicating increased arterial stiffness in patients with impaired glucose metabolism[103]. In a 2015 meta-analysis by Prenner *et al*[104], increased arterial stiffness (in most studies determined with cfPWV and AIx) correlated with higher mortality and a more advanced target organ damage (nephropathy, neuropathy and retinopathy) in diabetic patients. Laugesen *et al*[105] demonstrated that female patients with DM have the lowest values of subendocardial viability ratio (SEVR) compared to men with and without DM, indicating impaired subendocardial perfusion and endothelial dysfunction in this population.

Briet *et al*[106] performed a study on 95 patients with CKD (GFR measured with ^{51}Cr-EDTA clearance; 11% diabetics) and 121 hypertensive patients without CKD (GFR estimated with the use of Modification Diet in Renal Disease equation; 5% diabetics). They found that patients with CKD presented with increased arterial stiffness, determined by higher values of cfPWV. They explained their findings by the higher presence of DM and other traditional and non-traditional atherosclerosis risk factors in the CKD group. According to Sedaghat *et al*[107], the correlation between CKD and arterial stiffness is reciprocal, suggesting that besides being the result of CKD, increased arterial stiffness can lead to a faster CKD progression as well. A similar finding was found in a study by Fountoulakis *et al*[108], which was performed on diabetic patients with CKD. Proteinuria has also been linked to higher cfPWV and reduced SEVR and ejection duration[109,110].

Di Micco *et al*[111] performed a prospective, 3-year study involving 212 patients with CKD stages 3 and 4. During the study period, 34 patients died, 29 of them due to cardiovascular causes. Patients with lower SEVR had higher mortality. Post-mortem evaluation showed a higher degree of coronary artery calcification and a larger myocardial mass in patients with previously lower values of SEVR. Ekart *et al*[112] performed a study on non-dialysis CKD patients (27% diabetics) and found that SEVR < 130% predicted fatal and non-fatal cardiovascular events.

In a study by Kimoto *et al*[113], the authors found that the degree of CKD-associated increase in arterial stiffness varies among arterial regions in type 2 DM and is predominantly increased in the aorta. This has clinical implications because aortic stiffness is a strong and independent predictor of cardiovascular death, as shown in ESRD, DM, hypertensive and elderly patients.

IMAGING TECHNIQUES

Echocardiography

Structural and functional changes of the heart muscle are pivotal in understanding the increased cardiovascular risk in patients with DM and CKD. Both entities are related to macrovascular and microvascular pathology, resulting in increased myocardial fibrosis and subsequently in systolic and diastolic dysfunction[114,115]. It appears as though these changes are even more pronounced in the uremic milieu and are most likely intensified through myocardial calcifications in CKD patients[116]. Several studies have shown an independent association between DM, LVH and reduced systolic function of the left ventricle, both of which are commonly linked to an increased likelihood of sudden cardiac death, mostly due to arrhythmias[114,117,118]. LVH is especially common in CKD patients as it is linked to common comorbidities in these patients (AH, DM) and to CKD-specific factors, for example, volume overload,

Table 1 Arterial stiffness parameters and their definitions[98,99]

Arterial stiffness parameter	Definition
cfPWV (m/s)	Pulse wave distance between two measuring sites (carotid and femoral artery) divided by pulse transit time (measured by electrocardiographic monitoring)
PP (mmHg)	Difference between systolic and diastolic pressure
AP (mmHg)	Difference between systolic and inflection pressure
AIx (%)	AP divided by PP
AIx@75 (%)	AIx adjusted for heart rate at 75 beats per minute
ED (ms)	Duration of left ventricular systolic ejection
EDI (%)	The ratio of the duration of systolic ejection to the total duration of the heart cycle
SEVR (%)	The diastolic area under the curve divided by the systolic area under the curve, derived from the pulse wave curve

cfPWV: Carotid-femoral pulse wave velocity; PP: Pulse pressure; AP: Augmentation pressure; AIx: Augmentation index; AIx@75: AIx adjusted for heart rate 75/min; ED: Ejection duration; EDI: Ejection duration index; SEVR: Subendocardial viability ratio.

hyperphosphatemia and elevated levels of FGF-23. The clinical significance of LVH is prognostically unfavorable and is linked to increased cardiovascular mortality in patients with different degrees of CKD, including those on hemodialysis[119,120]. An additional strength of echocardiography is shown in a study by Di Cori *et al*[121], in which the authors found important subclinical dysfunction in asymptomatic type 1 DM patients aged under 40 years by using strain, strain rate and integrated backscatter. A similar finding was demonstrated in a study by Ha *et al*[122], in which the authors presented the importance of tissue Doppler indexes for unmasking subclinical myocardial ischemia. The prevalence of diastolic dysfunction, left atrial fibrosis and left atrial enlargement is also higher in patients with DM and CKD. All of the mentioned changes are a reflection of structural myocardial disease and are markers of increased cardiovascular risk as well[123].

Radionuclide imaging

Regadenoson-stress single-photon emission computed tomography (SPECT) is particularly appealing for cardiovascular risk assessment in asymptomatic diabetic patients [124]. The cause of concern in this population is silent myocardial ischemia and the data on the prevalence of this condition have been disparate. In the prospective detection of ischemia in asymptomatic diabetics study, which included asymptomatic patients with type 2 DM, a 22% prevalence of any perfusion defect or left ventricle dysfunction by SPECT was detected, and in 6% of patients, a moderate to large myocardial ischemia was found[125]. In a more recent analysis of 1354 asymptomatic patients (302 without DM) a lower prevalence of myocardial ischemia was found (7.2%). An important finding of the study was a much higher prevalence of silent ischemia observed in diabetic patients (12.5% *vs* 5.6%)[126]. The Basel asymptomatic high-risk diabetes outcome trial prospectively recruited 400 asymptomatic patients with type 2 DM. In the study, nearly a quarter of asymptomatic patients had silent myocardial ischemia, which was associated with a worse outcome and a higher likelihood of major adverse cardiovascular events[127]. The yield of SPECT can be further improved by choosing patients with higher basal cardiovascular risk, especially patients with certain other comorbidities, such as CKD. According to studies, abnormal SPECT is a good indicator of future acute coronary events and cardiovascular disease in diabetic and non-diabetic CKD patients[128]. Conversely, a normal SPECT is associated with a fairly good cardiovascular outcome in CKD patients, but it should be noted that due to accelerated coronary atherosclerosis in patients with CKD stages 4 and 5, a normal SPECT testing is still linked to higher cardiovascular risk compared to patients with better renal function[129]. In these patients, continuous follow-up is pivotal in preventing major adverse cardiovascular events[130].

Carotid intima-media thickness

Carotid IMT is an ultrasound-based, non-invasive measurement of atherosclerosis burden. It is used to investigate the determinants and consequents of atherosclerosis

and has been used as a surrogate end-point and a therapeutic target in some clinical trials as well[131,132].

Several clinical studies have demonstrated the association between the presence of atherosclerotic plaques in the carotid arteries, increased carotid IMT and atherosclerosis in other vascular territories[133]. Bots *et al*[134] have shown that patients with an increased carotid IMT have a higher likelihood of having advanced atherosclerotic plaques in the abdominal aorta. In a study by Ogata *et al*[135], a significant correlation between left main coronary atherosclerosis (determined by intravascular coronary ultrasound) and increased carotid IMT was observed. A positive association was also found for patients with symptomatic CAD, cerebrovascular disease and patients with PAD[136]. A descriptive, cross-sectional study was performed by Gómez-Marcos *et al*[137] in which they found an increased carotid IMT in patients with DM compared to patients without DM, and the difference was even greater in patients with advanced age. Signs of carotid damage were found in 23% of patients with DM. In a study by Matsagoura *et al*[138] that included patients with type 2 DM, an increased carotid IMT was observed in patients with moderately increased albuminuria or proteinuria compared to patients without proteinuria.

Kota *et al*[139] demonstrated a higher risk for ischemic stroke in type 2 DM patients with increased thickness of carotid intima-media. Sunil Kumar *et al*[140] performed a study on 30 patients with ESRD in which they found increased carotid IMT in these patients. They found the measurements to be an easy, non-invasive, easily-reproducible and cost-effective investigation in assessing cardiovascular risk in patients with chronic kidney failure. According to a study by Ekart *et al*[141], carotid IMT correlated with higher blood pressure in hemodialysis patients. In a study by Lawal *et al*[142], carotid IMT correlated with many cardiovascular risk factors among CKD patients, serving as a potential surrogate marker for cardiovascular disease in these patients. Roumeliotis *et al*[143] performed a study on 142 diabetic patients with different stages of CKD. Patients with increased carotid IMT had higher all-cause and cardiovascular mortality and had a higher degree of atherosclerosis in other vascular territories, further confirming the important role of carotid IMT measurements in recognizing patients with higher cardiovascular risk.

Coronary artery calcium score

High coronary artery calcium (CAC) score is associated with advanced atherosclerosis and with a 4-10-fold increase in the incidence of cardiovascular disease, independent of other risk factors[144]. Diabetic patients harbor larger amounts of CAC than non-diabetic patients of similar age[145]. Additionally, asymptomatic diabetic patients have a similar CAC than non-diabetic patients with known CAD[146]. In the coronary artery calcification in type 1 diabetes study, 656 adult patients with type 1 DM had higher CAC compared to 764 age- and sex-matched individuals without DM, with no differences between genders. Extensive vascular calcifications were registered even in younger patients with type 1 DM (17-28-years-old)[147]. The extent of CAC has also shown an important positive association with SPECT-registered myocardial ischemia, cardiovascular events and mortality[148,149]. Anand *et al*[150] followed 392 patients with type 2 DM and found that CAC progression was among the best predictors of increased cardiovascular risk.

Patients with CKD exhibit a higher prevalence of vascular calcification than the general population. In a 10-year prospective study on 137 CKD patients, the authors found that severe CAC was an important predictor of cardiovascular mortality[151]. Krajnc *et al*[152] compared CAC score between patients on hemodialysis and diabetic patients without renal involvement and found higher CAC score in the hemodialysis group, with the difference between both groups especially evident in the very high risk CAC score category. Besides being an adverse prognostic sign, higher CAC has been associated with faster progression of CKD to ESRD[153]. According to a study by Cano-Megías *et al*[154], the synergistic effect of DM and CKD leads to an even higher CAC score, increased inflammation and higher mortality compared to patients without DM, showing the importance of higher CAC in cardiovascular risk assessment of diabetic CKD patients.

Coronary computed tomography angiography

Coronary computed tomography angiography (CTA) is a non-invasive imaging modality that provides a detailed and comprehensive evaluation of the presence and the extent of CAD. According to a study by de Araújo Gonçalves *et al*[155], DM is an independent predictor of CAD and is associated with a more advanced CAD and a higher prevalence of atherosclerotic plaques in every anatomical subset of coronary arteries, all evaluated with the use of coronary CTA. An important obstructive CAD

was observed even in asymptomatic diabetic patients[156]. An additional important advantage of coronary CTA was found in a study by Madaj *et al*[157] in which they studied the presence of CAD in younger patients with type 1 and 2 DM. They found that coronary CTA detects CAD even in patients with a normal CAC score, which can be explained by a higher percentage of non-calcified plaques in younger patients with diabetes, rendering the CAC score less useful in this context. In diabetic CKD patients, coronary CTA is useful in determining the extent of CAD and atherosclerotic plaque characteristics; in CKD patients, a trend towards non-obstructive calcified plaques has been noticed[158]. There is, however, a reason for cautious use of coronary CTA in patients with advanced stages of CKD because of the risk of contrast-induced nephropathy[159].

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (CMRI) provides important prognostic information and aids in risk stratification in most cardiovascular diseases. It is a non-invasive imaging modality that can visualize myocardial scarring, myocardial steatosis and triglyceride content, interstitial fibrosis and interstitial myocardial edema. It is also useful in the evaluation of coronary arteries, valvular pathology, and in the differentiation of cardiomyopathies[160]. Diabetic patients are at risk of developing severe cardiomyopathy, partly due to CAD and partly due to metabolic derangements. The presence of late gadolinium hyperenhancement as a marker of prior myocardial infarction is associated with a 4-fold increased risk of a major adverse cardiovascular event and with a 7-fold increased risk of mortality[161]. Late gadolinium hyperenhancement was demonstrated in 4.3% of asymptomatic type 1 DM patients in the diabetes control and complications trial/epidemiology of diabetes interventions and complications trial[162] and 17% of asymptomatic diabetic older patients in a community-based study in Iceland[163].

T1 mapping is useful in assessing the amount of extracellular volume and interstitial fibrosis. Wong *et al*[164] demonstrated high short-term mortality in non-diabetic patients with increased extracellular volume. Some studies have shown increased extracellular volume and diastolic dysfunction in diabetic patients as well[165,166]. By using spectroscopy, myocardial lipid content can be assessed, which is an important pathophysiological step in understanding left ventricular dysfunction in diabetic patients[167]. Adenosine stress MRI test has excellent characteristics for the detection of obstructive CAD and microvascular dysfunction in patients with DM[168] and CKD patients as well[169]. Myocardial fibrosis is a hallmark of progressive CKD, uremic cardiomyopathy and is a crucial cause of increased cardiovascular risk in these patients[170]. Concerns relating to an association between gadolinium contrast agents and nephrogenic systemic fibrosis have led to the use of lower doses, lower risk gadolinium agents and even native CMRI in CKD patients, which is an important step forward in the wider use of CMRI in this subgroup of patients[171]. In the Graham-Brown *et al*[172] study on hemodialysis patients, a native CMRI was performed. The authors found an increase in myocardial fibrosis and interstitial edema, mostly in the septal region of the heart. By applying a similar methodology, Rutherford *et al*[170] showed that these findings were independent of patient's fluid status. In a study by Edwards *et al*[173] on CKD patients stages 2-4, a low dose gadolinium method was used. They found increased interstitial fibrosis and myocardial dysfunction in these patients, and the finding was not dependent on blood pressure. CMRI has a proven role in the understanding of uremic cardiomyopathy, ventricular dysfunction, myocardial fibrosis and cardiovascular risk assessment in patients with early and advanced CKD[171].

EPIGENETIC MARKERS

Epigenetics is the study of gene expression and involves phenotypic changes without changes in genotype. Several studies have confirmed that epigenetic regulations are crucial in the pathogenesis of atherosclerotic plaque formation, vessel wall inflammation and plaque rupture, in a cell-type and stage-specific manner[174,175]. Three epigenetic mechanisms are important in atherosclerosis: DNA methylation, post-translational modification of histones and the activity of non-coding ribonucleic acids (RNAs), most commonly long non-coding RNAs and micro RNAs (miR)[176]. The expression of non-coding RNAs can be measured in peripheral blood, urine and saliva using microarrays, indicating the potential of these markers as diagnostic tools and future therapeutic targets[177,178].

Several long non-coding RNAs are linked to LVH, myocardial infarction, heart failure and mortality[179]. Polymorphisms and increased expression of miR-124a, miR-375 and miR-146a are associated with obesity, insulin resistance and increased incidence of type 2 DM[180,181]. miR-27 exerts negative effects on adipogenesis and is associated with decreased incidence of DM[182]. In a study by Buraczynska *et al*[183], miR-196a2 has been linked to an increased likelihood of cardiovascular events in diabetic patients. miR-4513 is overly expressed in patients with metabolic syndrome and DM and is associated with major cardiovascular events, especially acute coronary syndrome[184]. Endothelial dysfunction is an important factor in CKD patients and according to a study by Kétszeri *et al*[178], miR-142-3p expression is linked to preventing endothelial dysfunction.

Non-coding RNAs are a marker of increased cardiovascular risk and can be employed to determine the risk of developing and/or progressing CKD in patients with DM. According to studies, several long non-coding RNAs (ERBB4-IR, MGC, ENSMUST00000147869) are centrally involved in the development and progression of CKD in patients with DM, either *via* direct pathogenic roles or as indirect mediators of different nephropathic and profibrotic pathways[185]. The upregulation of profibrotic miR-192 and miR-21 was confirmed to be linked to renal fibrosis, development and progression of CKD in diabetic patients[186]. Vice versa, reduced expression of anti-fibrotic miR-29 and miR-200 was also associated with more advanced kidney disease [187,188]. The downregulation of miR-30s, miR-124 and miR-93 has been shown to lead to increased podocyte injury and proteinuria[186]. In a study by Abdelsalam *et al* [189], miR-451 is a highly specific marker of CKD chronicity in patients with DM. In a study by Fourdinier *et al*[190], decreased serum levels of miR-126 and miR-223 were found in patients with advanced CKD with DM and were linked to increased mortality due to cardiovascular causes. It appears that the potential of non-coding RNAs is vast but currently of limited clinical importance due to high cost, limited availability and non-specificity of different molecules[176].

CONCLUSION

Due to the increasing burden of DM and CKD, the prevalence of the cardiovascular disease will continue to rise. To reduce morbidity, mortality and socioeconomic burden of these patients, immediate cardiovascular risk assessment is pivotal. Fulminant atherosclerosis, a hallmark of diabetic patients with CKD is a complex process, involving the interplay between traditional and non-traditional, CKD-specific risk factors, culminating in endothelial dysfunction, inflammation, plaque formation and ultimately target organ ischemia and damage. Due to the multifaceted process, it appears that a multimarker approach should be used to recognize patients with the highest risk for cardiovascular events. In the future, more attention should be given to the decrease in prevalence of DM and prevention of CKD development in diabetic patients.

REFERENCES

1 **Lin X**, Xu Y, Pan X, Xu J, Ding Y, Sun X, Song X, Ren Y, Shan PF. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Sci Rep* 2020; **10**: 14790 [PMID: 32901098 DOI: 10.1038/s41598-020-71908-9]

2 **Yang JJ**, Yu D, Wen W, Saito E, Rahman S, Shu XO, Chen Y, Gupta PC, Gu D, Tsugane S, Xiang YB, Gao YT, Yuan JM, Tamakoshi A, Irie F, Sadakane A, Tomata Y, Kanemura S, Tsuji I, Matsuo K, Nagata C, Chen CJ, Koh WP, Shin MH, Park SK, Wu PE, Qiao YL, Pednekar MS, He J, Sawada N, Li HL, Gao J, Cai H, Wang R, Sairenchi T, Grant E, Sugawara Y, Zhang S, Ito H, Wada K, Shen CY, Pan WH, Ahn YO, You SL, Fan JH, Yoo KY, Ashan H, Chia KS, Boffetta P, Inoue M, Kang D, Potter JD, Zheng W. Association of Diabetes With All-Cause and Cause-Specific Mortality in Asia: A Pooled Analysis of More Than 1 Million Participants. *JAMA Netw Open* 2019; **2**: e192696 [PMID: 31002328 DOI: 10.1001/jamanetworkopen.2019.2696]

3 **GBD Chronic Kidney Disease Collaboration**. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2020; **395**: 709-733 [PMID: 32061315 DOI: 10.1016/S0140-6736(20)30045-3]

4 **Carney EF**. The impact of chronic kidney disease on global health. *Nat Rev Nephrol* 2020; **16**: 251 [PMID: 32144399 DOI: 10.1038/s41581-020-0268-7]

5 **Lovre D**, Shah S, Sihota A, Fonseca VA. Managing Diabetes and Cardiovascular Risk in Chronic Kidney Disease Patients. *Endocrinol Metab Clin North Am* 2018; **47**: 237-257 [PMID: 29407054

DOI: 10.1016/j.ecl.2017.10.006]
6 **Suckling R**, Gallagher H. Chronic kidney disease, diabetes mellitus and cardiovascular disease: risks and commonalities. *J Ren Care* 2012; **38** Suppl 1: 4-11 [PMID: 22348359 DOI: 10.1111/j.1755-6686.2012.00274.x]
7 **Rule AD**, Gussak HM, Pond GR, Bergstralh EJ, Stegall MD, Cosio FG, Larson TS. Measured and estimated GFR in healthy potential kidney donors. *Am J Kidney Dis* 2004; **43**: 112-119 [PMID: 14712434 DOI: 10.1053/j.ajkd.2003.09.026]
8 **Go AS**, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**: 1296-1305 [PMID: 15385656 DOI: 10.1056/nejmoa041031]
9 **Wu J**, Geng J, Liu L, Teng W, Chen L. The Relationship between Estimated Glomerular Filtration Rate and Diabetic Retinopathy. *J Ophthalmol* 2015; **2015**: 326209 [PMID: 25866672 DOI: 10.1155/2015/326209]
10 **Babaliche P**, Nadpara RA, Maldar A. Association Between Estimated Glomerular Filtration Rate and Microvascular Complications in Type II Diabetes Mellitus Patients: A 1-Year Cross-Sectional Study. *J Natl Med Assoc* 2019; **111**: 83-87 [PMID: 30032867 DOI: 10.1016/j.jnma.2018.06.003]
11 **Dupuis ME**, Nadeau-Fredette AC, Madore F, Agharazii M, Goupil R. Association of Glomerular Hyperfiltration and Cardiovascular Risk in Middle-Aged Healthy Individuals. *JAMA Netw Open* 2020; **3**: e202377 [PMID: 32275320 DOI: 10.1001/jamanetworkopen.2020.2377]
12 **Groop PH**, Thomas MC, Moran JL, Wadèn J, Thorn LM, Mäkinen VP, Rosengård-Bärlund M, Saraheimo M, Hietala K, Heikkilä O, Forsblom C; FinnDiane Study Group. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. *Diabetes* 2009; **58**: 1651-1658 [PMID: 19401416 DOI: 10.2337/db08-1543]
13 **Davis TM**, Chubb SA, Davis WA. The relationship between estimated glomerular filtration rate trajectory and all-cause mortality in type 2 diabetes: the Fremantle Diabetes Study. *Eur J Endocrinol* 2016; **175**: 273-285 [PMID: 27418062 DOI: 10.1530/EJE-16-0327]
14 **Luo Y**, Wang X, Wang Y, Wang C, Wang H, Wang D, Liu L, Jia Q, Liu G, Zhao X, Wang Y; CNSR Investigators. Association of glomerular filtration rate with outcomes of acute stroke in type 2 diabetic patients: results from the China National Stroke Registry. *Diabetes Care* 2014; **37**: 173-179 [PMID: 24009297 DOI: 10.2337/dc13-1931]
15 **Eriksen BO**, Løchen ML, Arntzen KA, Bertelsen G, Eilertsen BA, von Hanno T, Herder M, Jenssen TG, Mathisen UD, Melsom T, Njølstad I, Solbu MD, Toft I, Mathiesen EB. Subclinical cardiovascular disease is associated with a high glomerular filtration rate in the nondiabetic general population. *Kidney Int* 2014; **86**: 146-153 [PMID: 24304885 DOI: 10.1038/ki.2013.470]
16 **Agrawal V**, Marinescu V, Agarwal M, McCullough PA. Cardiovascular implications of proteinuria: an indicator of chronic kidney disease. *Nat Rev Cardiol* 2009; **6**: 301-311 [PMID: 19352334 DOI: 10.1038/nrcardio.2009.11]
17 **de Zeeuw D**, Remuzzi G, Parving HH, Keane WF, Zhang Z, Shahinfar S, Snapinn S, Cooper ME, Mitch WE, Brenner BM. Albuminuria, a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy. *Circulation* 2004; **110**: 921-927 [PMID: 15302780 DOI: 10.1161/01.cir.0000139860.33974.28]
18 **Liu JE**, Robbins DC, Palmieri V, Bella JN, Roman MJ, Fabsitz R, Howard BV, Welty TK, Lee ET, Devereux RB. Association of albuminuria with systolic and diastolic left ventricular dysfunction in type 2 diabetes: the Strong Heart Study. *J Am Coll Cardiol* 2003; **41**: 2022-2028 [PMID: 12798576 DOI: 10.1016/s0735-1097(03)00403-0]
19 **Wattanakit K**, Folsom AR, Criqui MH, Kramer HJ, Cushman M, Shea S, Hirsch AT. Albuminuria and peripheral arterial disease: results from the multi-ethnic study of atherosclerosis (MESA). *Atherosclerosis* 2008; **201**: 212-216 [PMID: 18281047 DOI: 10.1016/j.atherosclerosis.2007.12.044]
20 **Ninomiya T**, Perkovic V, Verdon C, Barzi F, Cass A, Gallagher M, Jardine M, Anderson C, Chalmers J, Craig JC, Huxley R. Proteinuria and stroke: a meta-analysis of cohort studies. *Am J Kidney Dis* 2009; **53**: 417-425 [PMID: 19070947 DOI: 10.1053/j.ajkd.2008.08.032]
21 **Gerstein HC**, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Hallé JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S; HOPE Study Investigators. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 2001; **286**: 421-426 [PMID: 11466120 DOI: 10.1001/jama.286.4.421]
22 **Tuttle KR**, Puhlman ME, Cooney SK, Short R. Urinary albumin and insulin as predictors of coronary artery disease: An angiographic study. *Am J Kidney Dis* 1999; **34**: 918-925 [PMID: 10561150 DOI: 10.1016/s0272-6386(99)70051-x]
23 **Weir MR**. Microalbuminuria in type 2 diabetics: an important, overlooked cardiovascular risk factor. *J Clin Hypertens (Greenwich)* 2004; **6**: 134-41; quiz 142 [PMID: 15010646 DOI: 10.1111/j.1524-6175.2004.02524.x]
24 **Weir MR**. Microalbuminuria and cardiovascular disease. *Clin J Am Soc Nephrol* 2007; **2**: 581-590 [PMID: 17699466 DOI: 10.2215/cjn.03190906]
25 **Abbate M**, Zoja C, Remuzzi G. How does proteinuria cause progressive renal damage? *J Am Soc Nephrol* 2006; **17**: 2974-2984 [PMID: 17035611 DOI: 10.1681/asn.2006040377]
26 **Currie G**, Delles C. Proteinuria and its relation to cardiovascular disease. *Int J Nephrol Renovasc Dis* 2013; **7**: 13-24 [PMID: 24379690 DOI: 10.2147/IJNRD.S40522]
27 **Hojs R**, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. *Nephrol Dial*

Transplant 2006; **21**: 1855-1862 [PMID: 16524933 DOI: 10.1093/ndt/gfl073]
28 **Bevc S**, Hojs N, Knehtl M, Ekart R, Hojs R. Cystatin C as a predictor of mortality in elderly patients with chronic kidney disease. *Aging Male* 2019; **22**: 62-67 [PMID: 29912597 DOI: 10.1080/13685538.2018.1479386]
29 **Cepeda J**, Tranche-Iparraguirre S, Marín-Iranzo R, Fernández-Rodríguez E, Riesgo-García A, García-Casas J, Hevia-Rodríguez E. [Cystatin C and cardiovascular risk in the general population]. *Rev Esp Cardiol* 2010; **63**: 415-422 [PMID: 20334807 DOI: 10.1016/S1885-5857(10)70090-6]
30 **Correa S**, Morrow DA, Braunwald E, Davies RY, Goodrich EL, Murphy SA, Cannon CP, O'Donoghue ML. Cystatin C for Risk Stratification in Patients After an Acute Coronary Syndrome. *J Am Heart Assoc* 2018; **7**: e009077 [PMID: 30371283 DOI: 10.1161/JAHA.118.009077]
31 **Madero M**, Wassel CL, Peralta CA, Najjar SS, Sutton-Tyrrell K, Fried L, Canada R, Newman A, Shlipak MG, Sarnak MJ; Health ABC Study. Cystatin C associates with arterial stiffness in older adults. *J Am Soc Nephrol* 2009; **20**: 1086-1093 [PMID: 19357259 DOI: 10.1681/ASN.2008030318]
32 **Ix JH**, Shlipak MG, Chertow GM, Whooley MA. Association of cystatin C with mortality, cardiovascular events, and incident heart failure among persons with coronary heart disease: data from the Heart and Soul Study. *Circulation* 2007; **115**: 173-179 [PMID: 17190862 DOI: 10.1161/CIRCULATIONAHA.106.644286]
33 **Woitas RP**, Kleber ME, Meinitzer A, Grammer TB, Silbernagel G, Pilz S, Tomaschitz A, Weihrauch G, Dobnig H, März W, Scharnagl H. Cystatin C is independently associated with total and cardiovascular mortality in individuals undergoing coronary angiography. The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Atherosclerosis* 2013; **229**: 541-548 [PMID: 23706287 DOI: 10.1016/j.atherosclerosis.2013.04.027]
34 **Liu J**, Sukhova GK, Sun JS, Xu WH, Libby P, Shi GP. Lysosomal cysteine proteases in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1359-1366 [PMID: 15178558 DOI: 10.1161/01.atv.0000134530.27208.41]
35 **Kestenbaum B**, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 2005; **16**: 520-528 [PMID: 15615819 DOI: 10.1681/asn.2004070602]
36 **Isakova T**, Xie H, Yang W, Xie D, Anderson AH, Scialla J, Wahl P, Gutiérrez OM, Steigerwalt S, He J, Schwartz S, Lo J, Ojo A, Sondheimer J, Hsu CY, Lash J, Leonard M, Kusek JW, Feldman HI, Wolf M; Chronic Renal Insufficiency Cohort (CRIC) Study Group. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA* 2011; **305**: 2432-2439 [PMID: 21673295 DOI: 10.1001/jama.2011.826]
37 **Stöhr R**, Schuh A, Heine GH, Brandenburg V. FGF23 in Cardiovascular Disease: Innocent Bystander or Active Mediator? *Front Endocrinol (Lausanne)* 2018; **9**: 351 [PMID: 30013515 DOI: 10.3389/fendo.2018.00351]
38 **Judd SE**, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. *Am J Med Sci* 2009; **338**: 40-44 [PMID: 19593102 DOI: 10.1097/MAJ.0b013e3181aaee91]
39 **Khazai N**, Judd SE, Tangpricha V. Calcium and vitamin D: skeletal and extraskeletal health. *Curr Rheumatol Rep* 2008; **10**: 110-117 [PMID: 18460265 DOI: 10.1007/s11926-008-0020-y]
40 **Wahl P**, Xie H, Scialla J, Anderson CA, Bellovich K, Brecklin C, Chen J, Feldman H, Gutierrez OM, Lash J, Leonard MB, Negrea L, Rosas SE, Anderson AH, Townsend RR, Wolf M, Isakova T; Chronic Renal Insufficiency Cohort Study Group. Earlier onset and greater severity of disordered mineral metabolism in diabetic patients with chronic kidney disease. *Diabetes Care* 2012; **35**: 994-1001 [PMID: 22446176 DOI: 10.2337/dc11-2235]
41 **Libby P**. Inflammation in Atherosclerosis-No Longer a Theory. *Clin Chem* 2021; **67**: 131-142 [PMID: 33393629 DOI: 10.1093/clinchem/hvaa275]
42 **Koenig W**. High-sensitivity C-reactive protein and atherosclerotic disease: from improved risk prediction to risk-guided therapy. *Int J Cardiol* 2013; **168**: 5126-5134 [PMID: 23978367 DOI: 10.1016/j.ijcard.2013.07.113]
43 **Ridker PM**, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002; **347**: 1557-1565 [PMID: 12432042 DOI: 10.1056/nejmoa021993]
44 **Barzilay JI**, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, Tracy RP. The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. *Diabetes* 2001; **50**: 2384-2389 [PMID: 11574423 DOI: 10.2337/diabetes.50.10.2384]
45 **King DE**, Mainous AG 3rd, Buchanan TA, Pearson WS. C-reactive protein and glycemic control in adults with diabetes. *Diabetes Care* 2003; **26**: 1535-1539 [PMID: 12716818 DOI: 10.2337/diacare.26.5.1535]
46 **Kengne AP**, Batty GD, Hamer M, Stamatakis E, Czernichow S. Association of C-reactive protein with cardiovascular disease mortality according to diabetes status: pooled analyses of 25,979 participants from four U.K. prospective cohort studies. *Diabetes Care* 2012; **35**: 396-403 [PMID: 22210562 DOI: 10.2337/dc11-1588]
47 **Jager A**, van Hinsbergh VW, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arterioscler Thromb Vasc Biol* 1999; **19**: 3071-3078 [PMID: 10591689 DOI: 10.1161/01.atv.19.12.3071]
48 **Best LG**, Zhang Y, Lee ET, Yeh JL, Cowan L, Palmieri V, Roman M, Devereux RB, Fabsitz RR,

Tracy RP, Robbins D, Davidson M, Ahmed A, Howard BV. C-reactive protein as a predictor of cardiovascular risk in a population with a high prevalence of diabetes: the Strong Heart Study. *Circulation* 2005; **112**: 1289-1295 [PMID: 16116058 DOI: 10.1161/circulationaha.104.489260]

49 **Cox AJ**, Agarwal S, M Herrington D, Carr JJ, Freedman BI, Bowden DW. C-reactive protein concentration predicts mortality in type 2 diabetes: the Diabetes Heart Study. *Diabet Med* 2012; **29**: 767-770 [PMID: 22211818 DOI: 10.1111/j.1464-5491.2011.03560.x]

50 **Fu EL**, Franko MA, Obergfell A, Dekker FW, Gabrielsen A, Jernberg T, Carrero JJ. High-sensitivity C-reactive protein and the risk of chronic kidney disease progression or acute kidney injury in post-myocardial infarction patients. *Am Heart J* 2019; **216**: 20-29 [PMID: 31382219 DOI: 10.1016/j.ahj.2019.06.019]

51 **Chuang HH**, Lin RH, Li WC, Yeh WC, Lin YA, Chen JY. High-Sensitivity C-Reactive Protein Elevation Is Independently Associated with Subclinical Renal Impairment in the Middle-Aged and Elderly Population-A Community-Based Study in Northern Taiwan. *Int J Environ Res Public Health* 2020; **17** [PMID: 32823680 DOI: 10.3390/ijerph17165878]

52 **Sinha SK**, Nicholas SB, Sung JH, Correa A, Rajavashisth TB, Norris KC, Lee JE. hs-CRP Is Associated With Incident Diabetic Nephropathy: Findings From the Jackson Heart Study. *Diabetes Care* 2019; **42**: 2083-2089 [PMID: 31511234 DOI: 10.2337/dc18-2563]

53 **Jalal D**, Chonchol M, Etgen T, Sander D. C-reactive protein as a predictor of cardiovascular events in elderly patients with chronic kidney disease. *J Nephrol* 2012; **25**: 719-725 [PMID: 22038335 DOI: 10.5301/jn.5000047]

54 **Li WJ**, Chen XM, Nie XY, Zhang J, Cheng YJ, Lin XX, Wu SH. Cardiac troponin and C-reactive protein for predicting all-cause and cardiovascular mortality in patients with chronic kidney disease: a meta-analysis. *Clinics (Sao Paulo)* 2015; **70**: 301-311 [PMID: 26017799 DOI: 10.6061/clinics/2015(04)14]

55 **Liu L**, Gao B, Wang J, Yang C, Wu S, Wu Y, Chen S, Li Q, Zhang H, Wang G, Chen M, Zhao MH, Zhang L. Reduction in Serum High-Sensitivity C-Reactive Protein Favors Kidney Outcomes in Patients with Impaired Fasting Glucose or Diabetes. *J Diabetes Res* 2020; **2020**: 2720905 [PMID: 32587865 DOI: 10.1155/2020/2720905]

56 **Bavbek N**, Kargili A, Kaftan O, Karakurt F, Kosar A, Akcay A. Elevated concentrations of soluble adhesion molecules and large platelets in diabetic patients: are they markers of vascular disease and diabetic nephropathy? *Clin Appl Thromb Hemost* 2007; **13**: 391-397 [PMID: 17911190 DOI: 10.1177/1076029607303615]

57 **Clausen P**, Jacobsen P, Rossing K, Jensen JS, Parving HH, Feldt-Rasmussen B. Plasma concentrations of VCAM-1 and ICAM-1 are elevated in patients with Type 1 diabetes mellitus with microalbuminuria and overt nephropathy. *Diabet Med* 2000; **17**: 644-649 [PMID: 11051283 DOI: 10.1046/j.1464-5491.2000.00347.x]

58 **Güler S**, Cakir B, Demirbas B, Yönem A, Odabasi E, Onde U, Aykut O, Gürsoy G. Plasma soluble intercellular adhesion molecule 1 Levels are increased in type 2 diabetic patients with nephropathy. *Horm Res* 2002; **58**: 67-70 [PMID: 12207164 DOI: 10.1159/000064664]

59 **Becker A**, van Hinsbergh VW, Jager A, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Why is soluble intercellular adhesion molecule-1 related to cardiovascular mortality? *Eur J Clin Invest* 2002; **32**: 1-8 [PMID: 11851720 DOI: 10.1046/j.1365-2362.2002.00919.x]

60 **Kawamura T**, Umemura T, Kanai A, Uno T, Matsumae H, Sano T, Sakamoto N, Sakakibara T, Nakamura J, Hotta N. The incidence and characteristics of silent cerebral infarction in elderly diabetic patients: association with serum-soluble adhesion molecules. *Diabetologia* 1998; **41**: 911-917 [PMID: 9726593 DOI: 10.1007/s001250051007]

61 **Otsuki M**, Hashimoto K, Morimoto Y, Kishimoto T, Kasayama S. Circulating vascular cell adhesion molecule-1 (VCAM-1) in atherosclerotic NIDDM patients. *Diabetes* 1997; **46**: 2096-2101 [PMID: 9392502 DOI: 10.2337/diab.46.12.2096]

62 **Bevc S**, Sabic S, Hojs R. Atherosclerosis in hemodialysis patients--the role of microinflammation. *Ren Fail* 2008; **30**: 1012-1016 [PMID: 19016154 DOI: 10.1080/08860220802406385]

63 **Navarro JF**, Mora C. Diabetes, inflammation, proinflammatory cytokines, and diabetic nephropathy. *ScientificWorldJournal* 2006; **6**: 908-917 [PMID: 16906324 DOI: 10.1100/tsw.2006.179]

64 **Wada J**, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. *Clin Sci (Lond)* 2013; **124**: 139-152 [PMID: 23075333 DOI: 10.1042/CS20120198]

65 **Hojs R**, Ekart R, Bevc S, Hojs N. Biomarkers of Renal Disease and Progression in Patients with Diabetes. *J Clin Med* 2015; **4**: 1010-1024 [PMID: 26239462 DOI: 10.3390/jcm4051010]

66 **Nuhu F**, Bhandari S. Oxidative Stress and Cardiovascular Complications in Chronic Kidney Disease, the Impact of Anaemia. *Pharmaceuticals (Basel)* 2018; **11** [PMID: 30314359 DOI: 10.3390/ph11040103]

67 **Oguntibeju OO**. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol* 2019; **11**: 45-63 [PMID: 31333808]

68 **Vodošek Hojs N**, Bevc S, Ekart R, Hojs R. Oxidative Stress Markers in Chronic Kidney Disease with Emphasis on Diabetic Nephropathy. *Antioxidants (Basel)* 2020; **9** [PMID: 32992565 DOI: 10.3390/antiox9100925]

69 **Clement DL**, De Buyzere ML, De Bacquer DA, de Leeuw PW, Duprez DA, Fagard RH, Gheeraert PJ, Missault LH, Braun JJ, Six RO, Van Der Niepen P, O'Brien E; Office vs Ambulatory Pressure Study Investigators. Prognostic value of ambulatory blood-pressure recordings in patients with

treated hypertension. *N Engl J Med* 2003; **348**: 2407-2415 [PMID: 12802026 DOI: 10.1056/nejmoa022273]

70 **Hansen TW**, Jeppesen J, Rasmussen S, Ibsen H, Torp-Pedersen C. Ambulatory blood pressure monitoring and risk of cardiovascular disease: a population based study. *Am J Hypertens* 2006; **19**: 243-250 [PMID: 16500508 DOI: 10.1016/j.amjhyper.2005.09.018]

71 **Grezzana GB**, Moraes DW, Stein AT, Pellanda LC. Impact of Different Normality Thresholds for 24-hour ABPM at the Primary Health Care Level. *Arq Bras Cardiol* 2017; **108**: 143-148 [PMID: 28099585 DOI: 10.5935/abc.20160204]

72 **Iqbal P**, Stevenson L. Cardiovascular outcomes in patients with normal and abnormal 24-hour ambulatory blood pressure monitoring. *Int J Hypertens* 2010; **2011**: 786912 [PMID: 21151547 DOI: 10.4061/2011/786912]

73 **O'Brien E**, Asmar R, Beilin L, Imai Y, Mallion JM, Mancia G, Mengden T, Myers M, Padfield P, Palatini P, Parati G, Pickering T, Redon J, Staessen J, Stergiou G, Verdecchia P; European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens* 2003; **21**: 821-848 [PMID: 12714851 DOI: 10.1097/00004872-200305000-00001]

74 **Parati G**, Bilo G. Should 24-h ambulatory blood pressure monitoring be done in every patient with diabetes? *Diabetes Care* 2009; **32** Suppl 2: S298-S304 [PMID: 19875569 DOI: 10.2337/dc09-S326]

75 **Eguchi K**. Ambulatory blood pressure monitoring in diabetes and obesity-a review. *Int J Hypertens* 2011; **2011**: 954757 [PMID: 21629874 DOI: 10.4061/2011/954757]

76 **Manios E**, Stamatelopoulos K, Tsivgoulis G, Barlas G, Koroboki E, Tsagalis G, Michas F, Vemmos K, Zakopoulos N. Time rate of blood pressure variation: a new factor associated with coronary atherosclerosis. *J Hypertens* 2011; **29**: 1109-1114 [PMID: 21430560 DOI: 10.1097/HJH.0b013e3283454ff4]

77 **Velasquez MT**, Beddhu S, Nobakht E, Rahman M, Raj DS. Ambulatory Blood Pressure in Chronic Kidney Disease: Ready for Prime Time? *Kidney Int Rep* 2016; **1**: 94-104 [PMID: 28164170 DOI: 10.1016/j.ekir.2016.05.001]

78 **Davidson MB**, Hix JK, Vidt DG, Brotman DJ. Association of impaired diurnal blood pressure variation with a subsequent decline in glomerular filtration rate. *Arch Intern Med* 2006; **166**: 846-852 [PMID: 16636209 DOI: 10.1001/archinte.166.8.846]

79 **Agarwal R**, Andersen MJ. Prognostic importance of ambulatory blood pressure recordings in patients with chronic kidney disease. *Kidney Int* 2006; **69**: 1175-1180 [PMID: 16467785 DOI: 10.1038/sj.ki.5000247]

80 **Gabbai FB**, Rahman M, Hu B, Appel LJ, Charleston J, Contreras G, Faulkner ML, Hiremath L, Jamerson KA, Lea JP, Lipkowitz MS, Pogue VA, Rostand SG, Smogorzewski MJ, Wright JT, Greene T, Gassman J, Wang X, Phillips RA; African American Study of Kidney Disease and Hypertension (AASK) Study Group. Relationship between ambulatory BP and clinical outcomes in patients with hypertensive CKD. *Clin J Am Soc Nephrol* 2012; **7**: 1770-1776 [PMID: 22935847 DOI: 10.2215/CJN.11301111]

81 **Wang C**, Zhang J, Deng W, Gong W, Liu X, Ye Z, Peng H, Lou T. Nighttime Systolic Blood-Pressure Load Is Correlated with Target-Organ Damage Independent of Ambulatory Blood-Pressure Level in Patients with Non-Diabetic Chronic Kidney Disease. *PLoS One* 2015; **10**: e0131546 [PMID: 26186336 DOI: 10.1371/journal.pone.0131546]

82 **Amar J**, Vernier I, Rossignol E, Bongard V, Arnaud C, Conte JJ, Salvador M, Chamontin B. Nocturnal blood pressure and 24-hour pulse pressure are potent indicators of mortality in hemodialysis patients. *Kidney Int* 2000; **57**: 2485-2491 [PMID: 10844617 DOI: 10.1046/j.1523-1755.2000.00107.x]

83 **Liu M**, Takahashi H, Morita Y, Maruyama S, Mizuno M, Yuzawa Y, Watanabe M, Toriyama T, Kawahara H, Matsuo S. Non-dipping is a potent predictor of cardiovascular mortality and is associated with autonomic dysfunction in haemodialysis patients. *Nephrol Dial Transplant* 2003; **18**: 563-569 [PMID: 12584280 DOI: 10.1093/ndt/18.3.563]

84 **Tripepi G**, Fagugli RM, Dattolo P, Parlongo G, Mallamaci F, Buoncristiani U, Zoccali C. Prognostic value of 24-hour ambulatory blood pressure monitoring and of night/day ratio in nondiabetic, cardiovascular events-free hemodialysis patients. *Kidney Int* 2005; **68**: 1294-1302 [PMID: 16105064 DOI: 10.1111/j.1523-1755.2005.00527.x]

85 **Catalano M**, Scandale G, Carzaniga G, Cinquini M, Minola M, Antoniazzi V, Dimitrov G, Carotta M. Aortic augmentation index in patients with peripheral arterial disease. *J Clin Hypertens (Greenwich)* 2014; **16**: 782-787 [PMID: 25228305 DOI: 10.1111/jch.12406]

86 **Ugwu E**, Anyanwu A, Olamoyegun M. Ankle brachial index as a surrogate to vascular imaging in evaluation of peripheral artery disease in patients with type 2 diabetes. *BMC Cardiovasc Disord* 2021; **21**: 10 [PMID: 33407158 DOI: 10.1186/s12872-020-01821-6]

87 **Li J**, Luo Y, Xu Y, Yang J, Zheng L, Hasimu B, Yu J, Hu D. Risk factors of peripheral arterial disease and relationship between low ankle - brachial index and mortality from all-cause and cardiovascular disease in Chinese patients with type 2 diabetes. *Circ J* 2007; **71**: 377-381 [PMID: 17322639 DOI: 10.1253/circj.71.377]

88 **Alves-Cabratosa L**, Comas-Cufí M, Ponjoan A, Garcia-Gil M, Martí-Lluch R, Blanch J, Elosua-Bayes M, Parramon D, Camós L, Guzmán L, Ramos R. Levels of ankle-brachial index and the risk of diabetes mellitus complications. *BMJ Open Diabetes Res Care* 2020; **8** [PMID: 32144131 DOI: 10.1136/bmjdrc-2019-000977]

89 **Cosentino F**, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Jüni P, Lettino M, Marx N, Mellbin LG, Östgren CJ, Rocca B, Roffi M, Sattar N, Seferović PM, Sousa-Uva M, Valensi P, Wheeler DC; ESC Scientific Document Group. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* 2020; **41**: 255-323 [PMID: 31497854 DOI: 10.1093/eurheartj/ehz486]

90 **Ix JH**, Katz R, De Boer IH, Kestenbaum BR, Allison MA, Siscovick DS, Newman AB, Sarnak MJ, Shlipak MG, Criqui MH. Association of chronic kidney disease with the spectrum of ankle brachial index the CHS (Cardiovascular Health Study). *J Am Coll Cardiol* 2009; **54**: 1176-1184 [PMID: 19761940 DOI: 10.1016/j.jacc.2009.06.017]

91 **Nishimura H**, Miura T, Minamisawa M, Ueki Y, Abe N, Hashizume N, Mochidome T, Harada M, Shimizu K, Shoin W, Yoshie K, Oguchi Y, Ebisawa S, Motoki H, Izawa A, Koyama J, Ikeda U, Kuwahara K. Clinical Characteristics and Outcomes of Patients with High Ankle-Brachial Index from the IMPACT-ABI Study. *PLoS One* 2016; **11**: e0167150 [PMID: 27880852 DOI: 10.1371/journal.pone.0167150]

92 **Arroyo D**, Betriu A, Valls J, Gorriz JL, Pallares V, Abajo M, Gracia M, Valdivielso JM, Fernandez E; Investigators from the NEFRONA study. Factors influencing pathological ankle-brachial index values along the chronic kidney disease spectrum: the NEFRONA study. *Nephrol Dial Transplant* 2017; **32**: 513-520 [PMID: 27190385 DOI: 10.1093/ndt/gfw039]

93 **Chen FA**, Yang CY, Yang WC, Chen JY, Ng YY, Li SY, Liu WS, Cheng ST, Wang YJ, Lin CC. Ankle-brachial index is a powerful predictor of renal outcome and cardiovascular events in patients with chronic kidney disease. *ScientificWorldJournal* 2012; **2012**: 238494 [PMID: 22272169 DOI: 10.1100/2012/238494]

94 **Ix JH**, Katz R, Kestenbaum B, Fried LF, Kramer H, Stehman-Breen C, Shlipak MG. Association of mild to moderate kidney dysfunction and coronary calcification. *J Am Soc Nephrol* 2008; **19**: 579-585 [PMID: 18235089 DOI: 10.1681/ASN.2007070765]

95 **Bevc S**, Purg D, Knehtl M, Hren M, Turnšek N, Hojs N, Zorman T, Dvoršak B, Ekart R, Hojs R. Ankle-Brachial Index and Long-Term (10 Years) Survival of Nondiabetic Hemodialysis Patients. *Ther Apher Dial* 2016; **20**: 277-280 [PMID: 27312915 DOI: 10.1111/1744-9987.12437]

96 **Piko N**, Bevc S, Hojs R, Naji FH, Ekart R. The association between pulse wave analysis, carotid-femoral pulse wave velocity and peripheral arterial disease in patients with ischemic heart disease. *BMC Cardiovasc Disord* 2021; **21**: 33 [PMID: 33441117 DOI: 10.1186/s12872-021-01859-0]

97 **Vlachopoulos C**, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; **55**: 1318-1327 [PMID: 20338492 DOI: 10.1016/j.jacc.2009.10.061]

98 **Doupis J**, Papanas N, Cohen A, McFarlan L, Horton E. Pulse Wave Analysis by Applanation Tonometry for the Measurement of Arterial Stiffness. *Open Cardiovasc Med J* 2016; **10**: 188-195 [PMID: 27651842 DOI: 10.2174/1874192401610010188]

99 **Butlin M**, Qasem A. Large Artery Stiffness Assessment Using SphygmoCor Technology. *Pulse (Basel)* 2017; **4**: 180-192 [PMID: 28229053 DOI: 10.1159/000452448]

100 **Weber T**, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, Eber B. Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation* 2004; **109**: 184-189 [PMID: 14662706 DOI: 10.1161/01.cir.0000105767.94169.e3]

101 **Prskalo Z**, Brizić I, Markota D, Markota I, Boban M, Tomic M, Starcevic B. Arterial stiffness in patients with coronary artery disease: relation with in-stent restenosis following percutaneous coronary intervention. *BMC Cardiovasc Disord* 2016; **16**: 128 [PMID: 27266698 DOI: 10.1186/s12872-016-0305-4]

102 **Mattace-Raso FU**, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* 2006; **113**: 657-663 [PMID: 16461838 DOI: 10.1161/circulationaha.105.555235]

103 **Schram MT**, Henry RM, van Dijk RA, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Westerhof N, Stehouwer CD. Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. *Hypertension* 2004; **43**: 176-181 [PMID: 14698999 DOI: 10.1161/01.hyp.0000111829.46090.92]

104 **Prenner SB**, Chirinos JA. Arterial stiffness in diabetes mellitus. *Atherosclerosis* 2015; **238**: 370-379 [PMID: 25558032 DOI: 10.1016/j.atherosclerosis.2014.12.023]

105 **Laugesen E**, Høyem P, Fleischer J, Kumarathas I, Knudsen ST, Hansen KW, Christiansen JS, Hansen TK, Poulsen PL. Reduced Subendocardial Viability Ratio Is Associated With Unfavorable Cardiovascular Risk Profile in Women With Short Duration of Type 2 Diabetes. *Am J Hypertens* 2016; **29**: 1165-1172 [PMID: 27405963 DOI: 10.1093/ajh/hpw066]

106 **Briet M**, Bozec E, Laurent S, Fassot C, London GM, Jacquot C, Froissart M, Houillier P, Boutouyrie P. Arterial stiffness and enlargement in mild-to-moderate chronic kidney disease. *Kidney Int* 2006; **69**: 350-357 [PMID: 16408126 DOI: 10.1038/sj.ki.5000047]

107 **Sedaghat S**, Mattace-Raso FU, Hoorn EJ, Uitterlinden AG, Hofman A, Ikram MA, Franco OH, Dehghan A. Arterial Stiffness and Decline in Kidney Function. *Clin J Am Soc Nephrol* 2015; **10**: 2190-2197 [PMID: 26563380 DOI: 10.2215/CJN.03000315]

108 **Fountoulakis N**, Thakrar C, Patel K, Viberti G, Gnudi L, Karalliedde J. Increased Arterial Stiffness is an Independent Predictor of Renal Function Decline in Patients With Type 2 Diabetes Mellitus

Younger Than 60 Years. *J Am Heart Assoc* 2017; **6** [PMID: 28360227 DOI: 10.1161/jaha.116.004934]

109 **Weir MR**, Townsend RR, Fink JC, Teal V, Anderson C, Appel L, Chen J, He J, Litbarg N, Ojo A, Rahman M, Rosen L, Sozio SM, Steigerwalt S, Strauss L, Joffe MM. Hemodynamic correlates of proteinuria in chronic kidney disease. *Clin J Am Soc Nephrol* 2011; **6**: 2403-2410 [PMID: 21852669 DOI: 10.2215/CJN.01670211]

110 **Ekart R**, Bevc S, Hojs N, Knehtl M, Dvoršak B, Hojs R. Albuminuria is Associated With Subendocardial Viability Ratio in Chronic Kidney Disease Patients. *Kidney Blood Press Res* 2015; **40**: 565-574 [PMID: 26517083 DOI: 10.1159/000368532]

111 **Di Micco L**, Salvi P, Bellasi A, Sirico ML, Di Iorio B. Subendocardial viability ratio predicts cardiovascular mortality in chronic kidney disease patients. *Blood Purif* 2013; **36**: 26-28 [PMID: 23735512 DOI: 10.1159/000350582]

112 **Ekart R**, Bevc S, Hojs N, Hojs R. Derived Subendocardial Viability Ratio and Cardiovascular Events in Patients with Chronic Kidney Disease. *Cardiorenal Med* 2019; **9**: 41-50 [PMID: 30408781 DOI: 10.1159/000493512]

113 **Kimoto E**, Shoji T, Shinohara K, Hatsuda S, Mori K, Fukumoto S, Koyama H, Emoto M, Okuno Y, Nishizawa Y. Regional arterial stiffness in patients with type 2 diabetes and chronic kidney disease. *J Am Soc Nephrol* 2006; **17**: 2245-2252 [PMID: 16837632 DOI: 10.1681/asn.2005101038]

114 **Galderisi M**. Diastolic dysfunction and diabetic cardiomyopathy: evaluation by Doppler echocardiography. *J Am Coll Cardiol* 2006; **48**: 1548-1551 [PMID: 17045886 DOI: 10.1016/j.jacc.2006.07.033]

115 **Budoff MJ**, Raggi P, Beller GA, Berman DS, Druz RS, Malik S, Rigolin VH, Weigold WG, Soman P; Imaging Council of the American College of Cardiology. Noninvasive Cardiovascular Risk Assessment of the Asymptomatic Diabetic Patient: The Imaging Council of the American College of Cardiology. *JACC Cardiovasc Imaging* 2016; **9**: 176-192 [PMID: 26846937 DOI: 10.1016/j.jcmg.2015.11.011]

116 **Marcassi AP**, Yasbek DC, Pestana JO, Fachini FC, De Lira Filho EB, Cassiolato JL, Canziani ME. Ventricular arrhythmia in incident kidney transplant recipients: prevalence and associated factors. *Transpl Int* 2011; **24**: 67-72 [PMID: 20723177 DOI: 10.1111/j.1432-2277.2010.01149.x]

117 **Galderisi M**, Anderson KM, Wilson PW, Levy D. Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (the Framingham Heart Study). *Am J Cardiol* 1991; **68**: 85-89 [PMID: 2058564 DOI: 10.1016/0002-9149(91)90716-x]

118 **Devereux RB**, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, Fabsitz RR, Robbins D, Rhoades ER, Howard BV. Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation* 2000; **101**: 2271-2276 [PMID: 10811594 DOI: 10.1161/01.cir.101.19.2271]

119 **Pecoits-Filho R**, Barberato SH. Echocardiography in chronic kidney disease: diagnostic and prognostic implications. *Nephron Clin Pract* 2010; **114**: c242-c247 [PMID: 20090365 DOI: 10.1159/000276575]

120 **Ekart R**, Kanič V, Pečovnik Balon B, Bevc S, Hojs R. Prognostic value of 48-hour ambulatory blood pressure measurement and cardiovascular mortality in hemodialysis patients. *Kidney Blood Press Res* 2012; **35**: 326-331 [PMID: 22398387 DOI: 10.1159/000336357]

121 **Di Cori A**, Di Bello V, Miccoli R, Talini E, Palagi C, Delle Donne MG, Penno G, Nardi C, Bianchi C, Mariani M, Del Prato S, Balbarini A. Left ventricular function in normotensive young adults with well-controlled type 1 diabetes mellitus. *Am J Cardiol* 2007; **99**: 84-90 [PMID: 17196468 DOI: 10.1016/j.amjcard.2006.07.063]

122 **Ha JW**, Lee HC, Kang ES, Ahn CM, Kim JM, Ahn JA, Lee SW, Choi EY, Rim SJ, Oh JK, Chung N. Abnormal left ventricular longitudinal functional reserve in patients with diabetes mellitus: implication for detecting subclinical myocardial dysfunction using exercise tissue Doppler echocardiography. *Heart* 2007; **93**: 1571-1576 [PMID: 17449503 DOI: 10.1136/hrt.2006.101667]

123 **Boyer JK**, Thanigaraj S, Schechtman KB, Pérez JE. Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. *Am J Cardiol* 2004; **93**: 870-875 [PMID: 15050491 DOI: 10.1016/j.amjcard.2003.12.026]

124 **Hachamovitch R**, Kang X, Amanullah AM, Abidov A, Hayes SW, Friedman JD, Cohen I, Thomson LE, Germano G, Berman DS. Prognostic implications of myocardial perfusion single-photon emission computed tomography in the elderly. *Circulation* 2009; **120**: 2197-2206 [PMID: 19917890 DOI: 10.1161/CIRCULATIONAHA.108.817387]

125 **Wackers FJ**, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE; Detection of Ischemia in Asymptomatic Diabetics Investigators. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. *Diabetes Care* 2004; **27**: 1954-1961 [PMID: 15277423 DOI: 10.2337/diacare.27.8.1954]

126 **Malhotra S**, Sharma R, Kliner DE, Follansbee WP, Soman P. Relationship between silent myocardial ischemia and coronary artery disease risk factors. *J Nucl Cardiol* 2013; **20**: 731-738 [PMID: 23719838 DOI: 10.1007/s12350-013-9708-0]

127 **Zellweger MJ**, Maraun M, Osterhues HH, Keller U, Müller-Brand J, Jeger R, Pfister O, Burkard T, Eckstein F, von Felten S, Osswald S, Pfisterer M. Progression to overt or silent CAD in asymptomatic patients with diabetes mellitus at high coronary risk: main findings of the prospective multicenter BARDOT trial with a pilot randomized treatment substudy. *JACC Cardiovasc Imaging* 2014; **7**: 1001-1010 [PMID: 25240454 DOI: 10.1016/j.jcmg.2014.07.010]

128 **Rabbat CG**, Treleaven DJ, Russell JD, Ludwin D, Cook DJ. Prognostic value of myocardial perfusion studies in patients with end-stage renal disease assessed for kidney or kidney-pancreas transplantation: a meta-analysis. *J Am Soc Nephrol* 2003; **14**: 431-439 [PMID: 12538744 DOI: 10.1097/01.asn.0000047560.51444.3a]

129 **Marie PY**, Rossignol P. Stress myocardial perfusion gated-SPECT imaging in advanced chronic kidney disease. *J Nucl Cardiol* 2019; **26**: 1971-1973 [PMID: 30350266 DOI: 10.1007/s12350-018-1412-7]

130 **Furuhashi T**, Moroi M, Joki N, Hase H, Minakawa M, Masai H, Kunimasa T, Fukuda H, Sugi K. Prediction of cardiovascular events in pre-dialysis chronic kidney disease patients with normal SPECT myocardial perfusion imaging. *J Cardiol* 2014; **63**: 154-158 [PMID: 24001741 DOI: 10.1016/j.jjcc.2013.07.011]

131 **Centurión OA**. Carotid Intima-Media Thickness as a Cardiovascular Risk Factor and Imaging Pathway of Atherosclerosis. *Crit Pathw Cardiol* 2016; **15**: 152-160 [PMID: 27846007 DOI: 10.1097/hpc.0000000000000087]

132 **Hojs R**. Carotid intima-media thickness and plaques in hemodialysis patients. *Artif Organs* 2000; **24**: 691-695 [PMID: 11012538 DOI: 10.1046/j.1525-1594.2000.06466.x]

133 **Akazawa S**, Tojikubo M, Nakano Y, Nakamura S, Tamai H, Yonemoto K, Sadasima E, Kawasaki T, Koga N. Usefulness of carotid plaque (sum and maximum of plaque thickness) in combination with intima-media thickness for the detection of coronary artery disease in asymptomatic patients with diabetes. *J Diabetes Investig* 2016; **7**: 396-403 [PMID: 27330727 DOI: 10.1111/jdi.12403]

134 **Bots ML**, Witteman JC, Grobbee DE. Carotid intima-media wall thickness in elderly women with and without atherosclerosis of the abdominal aorta. *Atherosclerosis* 1993; **102**: 99-105 [PMID: 8257458 DOI: 10.1016/0021-9150(93)90088-c]

135 **Ogata T**, Yasaka M, Yamagishi M, Seguchi O, Nagatsuka K, Minematsu K. Atherosclerosis found on carotid ultrasonography is associated with atherosclerosis on coronary intravascular ultrasonography. *J Ultrasound Med* 2005; **24**: 469-474 [PMID: 15784765 DOI: 10.7863/jum.2005.24.4.469]

136 **Dijk JM**, van der Graaf Y, Bots ML, Grobbee DE, Algra A. Carotid intima-media thickness and the risk of new vascular events in patients with manifest atherosclerotic disease: the SMART study. *Eur Heart J* 2006; **27**: 1971-1978 [PMID: 16835264 DOI: 10.1093/eurheartj/ehl136]

137 **Gómez-Marcos MA**, Recio-Rodríguez JI, Rodríguez-Sánchez E, Patino-Alonso MC, Magallón-Botaya R, Martínez-Vizcaino V, Gómez Sánchez L, García-Ortiz L. [Carotid intima-media thickness in diabetics and hypertensive patients]. *Rev Esp Cardiol* 2011; **64**: 622-625 [PMID: 21440358 DOI: 10.1016/j.recesp.2010.10.025]

138 **Matsagoura M**, Andreadis E, Diamantopoulos EJ, Vassilopoulos C, Tentolouris N, Katsilambros N. Carotid intima-media thickness in patients with type 2 diabetes: the significance of microalbuminuria and different risk factors for atherosclerosis. *Diabetes Care* 2003; **26**: 2966 [PMID: 14514622 DOI: 10.2337/diacare.26.10.2966]

139 **Kota SK**, Mahapatra GB, Kota SK, Naveed S, Tripathy PR, Jammula S, Modi KD. Carotid intima media thickness in type 2 diabetes mellitus with ischemic stroke. *Indian J Endocrinol Metab* 2013; **17**: 716-722 [PMID: 23961492 DOI: 10.4103/2230-8210.113767]

140 **Sunil Kumar K**, Lakshmi AY, Srinivasa Rao PV, Das GC, Siva Kumar V. Carotid intima-media thickness in patients with end-stage renal disease. *Indian J Nephrol* 2009; **19**: 13-14 [PMID: 20352005 DOI: 10.4103/0971-4065.50674]

141 **Ekart R**, Hojs R, Pecovnik-Balon B, Bevc S, Dvorsak B. Blood pressure measurements and carotid intima media thickness in hemodialysis patients. *Ther Apher Dial* 2009; **13**: 288-293 [PMID: 19695061 DOI: 10.1111/j.1744-9987.2009.00726.x]

142 **Lawal OM**, Balogun MO, Akintomide AO, Ayoola OO, Mene-Afejuku TO, Ogunlade O, Okunola OO, Lawal AO, Akinsola A. Carotid Intima-Media Thickness: A Surrogate Marker for Cardiovascular Disease in Chronic Kidney Disease Patients. *Clin Med Insights Cardiol* 2019; **13**: 1179546819852941 [PMID: 31258338 DOI: 10.1177/1179546819852941]

143 **Roumeliotis A**, Roumeliotis S, Panagoutsos S, Theodoridis M, Argyriou C, Tavridou A, Georgiadis GS. Carotid intima-media thickness is an independent predictor of all-cause mortality and cardiovascular morbidity in patients with diabetes mellitus type 2 and chronic kidney disease. *Ren Fail* 2019; **41**: 131-138 [PMID: 30909780 DOI: 10.1080/0886022X.2019.1585372]

144 **Yeboah J**, Young R, McClelland RL, Delaney JC, Polonsky TS, Dawood FZ, Blaha MJ, Miedema MD, Sibley CT, Carr JJ, Burke GL, Goff DC Jr, Psaty BM, Greenland P, Herrington DM. Utility of Nontraditional Risk Markers in Atherosclerotic Cardiovascular Disease Risk Assessment. *J Am Coll Cardiol* 2016; **67**: 139-147 [PMID: 26791059 DOI: 10.1016/j.jacc.2015.10.058]

145 **Wong ND**, Sciammarella MG, Polk D, Gallagher A, Miranda-Peats L, Whitcomb B, Hachamovitch R, Friedman JD, Hayes S, Berman DS. The metabolic syndrome, diabetes, and subclinical atherosclerosis assessed by coronary calcium. *J Am Coll Cardiol* 2003; **41**: 1547-1553 [PMID: 12742296 DOI: 10.1016/s0735-1097(03)00193-1]

146 **Khaleeli E**, Peters SR, Bobrowsky K, Oudiz RJ, Ko JY, Budoff MJ. Diabetes and the associated incidence of subclinical atherosclerosis and coronary artery disease: Implications for management. *Am Heart J* 2001; **141**: 637-644 [PMID: 11275932 DOI: 10.1067/mhj.2001.113224]

147 **Dabelea D**, Kinney G, Snell-Bergeon JK, Hokanson JE, Eckel RH, Ehrlich J, Garg S, Hamman RF, Rewers M; Coronary Artery Calcification in Type 1 Diabetes Study. Effect of type 1 diabetes on the gender difference in coronary artery calcification: a role for insulin resistance? *Diabetes* 2003; **52**:

2833-2839 [PMID: 14578303 DOI: 10.2337/diabetes.52.11.2833]
148 **Berman DS**, Wong ND, Gransar H, Miranda-Peats R, Dahlbeck J, Hayes SW, Friedman JD, Kang X, Polk D, Hachamovitch R, Shaw L, Rozanski A. Relationship between stress-induced myocardial ischemia and atherosclerosis measured by coronary calcium tomography. *J Am Coll Cardiol* 2004; **44**: 923-930 [PMID: 15312881 DOI: 10.1016/j.jacc.2004.06.042]
149 **Mirbolouk M**, Kianoush S, Dardari Z, Miedema MD, Shaw LJ, Rumberger JA, Berman DS, Budoff MJ, Rozanski A, Al-Mallah MH, McEvoy JW, Nasir K, Blaha MJ. The association of coronary artery calcium score and mortality risk among smokers: The coronary artery calcium consortium. *Atherosclerosis* 2020; **294**: 33-40 [PMID: 31951880 DOI: 10.1016/j.atherosclerosis.2019.12.014]
150 **Anand DV**, Lim E, Darko D, Bassett P, Hopkins D, Lipkin D, Corder R, Lahiri A. Determinants of progression of coronary artery calcification in type 2 diabetes role of glycemic control and inflammatory/vascular calcification markers. *J Am Coll Cardiol* 2007; **50**: 2218-2225 [PMID: 18061069 DOI: 10.1016/j.jacc.2007.08.032]
151 **Cano-Megías M**, Guisado-Vasco P, Bouarich H, de Arriba-de la Fuente G, de Sequera-Ortiz P, Álvarez-Sanz C, Rodríguez-Puyol D. Coronary calcification as a predictor of cardiovascular mortality in advanced chronic kidney disease: a prospective long-term follow-up study. *BMC Nephrol* 2019; **20**: 188 [PMID: 31138150 DOI: 10.1186/s12882-019-1367-1]
152 **Krajnc M**, Pečovnik-Balon B, Hojs R, Rupreht M. Comparison of coronary artery calcification and some coronary artery calcification risk factors in patients on haemodialysis and in patients with type 2 diabetes. *J Int Med Res* 2011; **39**: 1006-1015 [PMID: 21819735 DOI: 10.1177/147323001103900336]
153 **Lamarche MC**, Hopman WM, Garland JS, White CA, Holden RM. Relationship of coronary artery calcification with renal function decline and mortality in predialysis chronic kidney disease patients. *Nephrol Dial Transplant* 2019; **34**: 1715-1722 [PMID: 30010904 DOI: 10.1093/ndt/gfy183]
154 **Cano-Megías M**, Bouarich H, Guisado-Vasco P, Pérez Fernández M, de Arriba-de la Fuente G, Álvarez-Sanz C, Rodríguez-Puyol D. Coronary artery calcification in patients with diabetes mellitus and advanced chronic kidney disease. *Endocrinol Diabetes Nutr* 2019; **66**: 297-304 [PMID: 30509882 DOI: 10.1016/j.endinu.2018.09.003]
155 **de Araújo Gonçalves P**, Garcia-Garcia HM, Carvalho MS, Dores H, Sousa PJ, Marques H, Ferreira A, Cardim N, Teles RC, Raposo L, Gabriel HM, Almeida M, Aleixo A, Carmo MM, Machado FP, Mendes M. Diabetes as an independent predictor of high atherosclerotic burden assessed by coronary computed tomography angiography: the coronary artery disease equivalent revisited. *Int J Cardiovasc Imaging* 2013; **29**: 1105-1114 [PMID: 23239217 DOI: 10.1007/s10554-012-0168-4]
156 **Kamimura M**, Moroi M, Isobe M, Hiroe M. Role of coronary CT angiography in asymptomatic patients with type 2 diabetes mellitus. *Int Heart J* 2012; **53**: 23-28 [PMID: 22398672 DOI: 10.1536/ihj.53.23]
157 **Madaj PM**, Budoff MJ, Li D, Tayek JA, Karlsberg RP, Karpman HL. Identification of noncalcified plaque in young persons with diabetes: an opportunity for early primary prevention of coronary artery disease identified with low-dose coronary computed tomographic angiography. *Acad Radiol* 2012; **19**: 889-893 [PMID: 22542200 DOI: 10.1016/j.acra.2012.03.013]
158 **Deng W**, Peng L, Yu J, Shuai T, Chen Z, Li Z. Characteristics of coronary artery atherosclerotic plaques in chronic kidney disease: evaluation with coronary CT angiography. *Clin Radiol* 2019; **74**: 731.e1-731. e9 [PMID: 31285036 DOI: 10.1016/j.crad.2019.06.003]
159 **Sarnak MJ**, Amann K, Bangalore S, Cavalcante JL, Charytan DM, Craig JC, Gill JS, Hlatky MA, Jardine AG, Landmesser U, Newby LK, Herzog CA, Cheung M, Wheeler DC, Winkelmayer WC, Marwick TH; Conference Participants. Chronic Kidney Disease and Coronary Artery Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2019; **74**: 1823-1838 [PMID: 31582143 DOI: 10.1016/j.jacc.2019.08.1017]
160 **Aljaroudi WA**, Flamm SD, Saliba W, Wilkoff BL, Kwon D. Role of CMR imaging in risk stratification for sudden cardiac death. *JACC Cardiovasc Imaging* 2013; **6**: 392-406 [PMID: 23473115 DOI: 10.1016/j.jcmg.2012.11.011]
161 **Manfrini O**, Russo V, Ciavarella A, Ceroni L, Montalti M, Fattori R. Coronary plaque quantification and composition in asymptomatic patients with type II diabetes mellitus. *J Cardiovasc Med (Hagerstown)* 2012; **13**: 423-431 [PMID: 22673024 DOI: 10.2459/JCM.0b013e32835593f9]
162 **Kwong RY**, Sattar H, Wu H, Vorobiof G, Gandla V, Steel K, Siu S, Brown KA. Incidence and prognostic implication of unrecognized myocardial scar characterized by cardiac magnetic resonance in diabetic patients without clinical evidence of myocardial infarction. *Circulation* 2008; **118**: 1011-1020 [PMID: 18725488 DOI: 10.1161/CIRCULATIONAHA.107.727826]
163 **Schelbert EB**, Cao JJ, Sigurdsson S, Aspelund T, Kellman P, Aletras AH, Dyke CK, Thorgeirsson G, Eiriksdottir G, Launer LJ, Gudnason V, Harris TB, Arai AE. Prevalence and prognosis of unrecognized myocardial infarction determined by cardiac magnetic resonance in older adults. *JAMA* 2012; **308**: 890-896 [PMID: 22948699 DOI: 10.1001/2012.jama.11089]
164 **Wong TC**, Piehler K, Meier CG, Testa SM, Klock AM, Aneizi AA, Shakesprere J, Kellman P, Shroff SG, Schwartzman DS, Mulukutla SR, Simon MA, Schelbert EB. Association between extracellular matrix expansion quantified by cardiovascular magnetic resonance and short-term mortality. *Circulation* 2012; **126**: 1206-1216 [PMID: 22851543 DOI: 10.1161/CIRCULATIONAHA.111.089409]
165 **Jellis C**, Wright J, Kennedy D, Sacre J, Jenkins C, Haluska B, Martin J, Fenwick J, Marwick TH. Association of imaging markers of myocardial fibrosis with metabolic and functional disturbances in

early diabetic cardiomyopathy. *Circ Cardiovasc Imaging* 2011; **4**: 693-702 [PMID: 21946703 DOI: 10.1161/CIRCIMAGING.111.963587]

166 **Rijzewijk LJ**, van der Meer RW, Smit JW, Diamant M, Bax JJ, Hammer S, Romijn JA, de Roos A, Lamb HJ. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol* 2008; **52**: 1793-1799 [PMID: 19022158 DOI: 10.1016/j.jacc.2008.07.062]

167 **van der Meer RW**, Lamb HJ, Smit JW, de Roos A. MR imaging evaluation of cardiovascular risk in metabolic syndrome. *Radiology* 2012; **264**: 21-37 [PMID: 22723560 DOI: 10.1148/radiol.12110772]

168 **Levelt E**, Piechnik SK, Liu A, Wijesurendra RS, Mahmod M, Ariga R, Francis JM, Greiser A, Clarke K, Neubauer S, Ferreira VM, Karamitsos TD. Adenosine stress CMR T1-mapping detects early microvascular dysfunction in patients with type 2 diabetes mellitus without obstructive coronary artery disease. *J Cardiovasc Magn Reson* 2017; **19**: 81 [PMID: 29070069 DOI: 10.1186/s12968-017-0397-8]

169 **Shah R**, Sree Raman K, Walls A, Woodman RJ, Faull R, Gleadle JM, Selvanayagam JB. Gadolinium-Free Cardiovascular Magnetic Resonance Stress T1 Mapping in Patients With Chronic Kidney Disease. *JACC Cardiovasc Imaging* 2019; **12**: 2083-2085 [PMID: 31202747 DOI: 10.1016/j.jcmg.2019.04.016]

170 **Rutherford E**, Talle MA, Mangion K, Bell E, Rauhalammi SM, Roditi G, McComb C, Radjenovic A, Welsh P, Woodward R, Struthers AD, Jardine AG, Patel RK, Berry C, Mark PB. Defining myocardial tissue abnormalities in end-stage renal failure with cardiac magnetic resonance imaging using native T1 mapping. *Kidney Int* 2016; **90**: 845-852 [PMID: 27503805 DOI: 10.1016/j.kint.2016.06.014]

171 **Edwards NC**, Moody WE, Chue CD, Ferro CJ, Townend JN, Steeds RP. Defining the natural history of uremic cardiomyopathy in chronic kidney disease: the role of cardiovascular magnetic resonance. *JACC Cardiovasc Imaging* 2014; **7**: 703-714 [PMID: 25034920 DOI: 10.1016/j.jcmg.2013.09.025]

172 **Graham-Brown MP**, March DS, Churchward DR, Stensel DJ, Singh A, Arnold R, Burton JO, McCann GP. Novel cardiac nuclear magnetic resonance method for noninvasive assessment of myocardial fibrosis in hemodialysis patients. *Kidney Int* 2016; **90**: 835-844 [PMID: 27633869 DOI: 10.1016/j.kint.2016.07.014]

173 **Edwards NC**, Moody WE, Yuan M, Hayer MK, Ferro CJ, Townend JN, Steeds RP. Diffuse interstitial fibrosis and myocardial dysfunction in early chronic kidney disease. *Am J Cardiol* 2015; **115**: 1311-1317 [PMID: 25769628 DOI: 10.1016/j.amjcard.2015.02.015]

174 **Valencia-Morales Mdel P**, Zaina S, Heyn H, Carmona FJ, Varol N, Sayols S, Condom E, Ramírez-Ruz J, Gomez A, Moran S, Lund G, Rodríguez-Ríos D, López-González G, Ramírez-Nava M, de la Rocha C, Sanchez-Flores A, Esteller M. The DNA methylation drift of the atherosclerotic aorta increases with lesion progression. *BMC Med Genomics* 2015; **8**: 7 [PMID: 25881171 DOI: 10.1186/s12920-015-0085-1]

175 **Cao J**, Ye B, Lin L, Tian L, Yang H, Wang C, Huang W, Huang Z. Curcumin Alleviates oxLDL Induced MMP-9 and EMMPRIN Expression through the Inhibition of NF-κB and MAPK Pathways in Macrophages. *Front Pharmacol* 2017; **8**: 62 [PMID: 28261097 DOI: 10.3389/fphar.2017.00062]

176 **Shirodkar AV**, Marsden PA. Epigenetics in cardiovascular disease. *Curr Opin Cardiol* 2011; **26**: 209-215 [PMID: 21415727 DOI: 10.1097/HCO.0b013e328345986e]

177 **Feinberg MW**, Moore KJ. MicroRNA Regulation of Atherosclerosis. *Circ Res* 2016; **118**: 703-720 [PMID: 26892968 DOI: 10.1161/CIRCRESAHA.115.306300]

178 **Kétszeri M**, Kirsch A, Frauscher B, Moschovaki-Filippidou F, Mooslechner AA, Kirsch AH, Schabhuettl C, Aringer I, Artinger K, Pregartner G, Ekart R, Breznik S, Hojs R, Goessler W, Schilcher I, Müller H, Obermayer-Pietsch B, Frank S, Rosenkranz AR, Eller P, Eller K. MicroRNA-142-3p improves vascular relaxation in uremia. *Atherosclerosis* 2019; **280**: 28-36 [PMID: 30453118 DOI: 10.1016/j.atherosclerosis.2018.11.024]

179 **Hobuß L**, Bär C, Thum T. Long Non-coding RNAs: At the Heart of Cardiac Dysfunction? *Front Physiol* 2019; **10**: 30 [PMID: 30761015 DOI: 10.3389/fphys.2019.00030]

180 **Li Y**, Li C, Yang M, Shi L, Tao W, Shen K, Li X, Wang X, Yang Y, Yao Y. Association of single nucleotide polymorphisms of miRNAs involved in the GLUT4 pathway in T2DM in a Chinese population. *Mol Genet Genomic Med* 2019; **7**: e907 [PMID: 31389668 DOI: 10.1002/mgg3.907]

181 **Erener S**, Mojibian M, Fox JK, Denroche HC, Kieffer TJ. Circulating miR-375 as a biomarker of β-cell death and diabetes in mice. *Endocrinology* 2013; **154**: 603-608 [PMID: 23321698 DOI: 10.1210/en.2012-1744]

182 **Ciccacci C**, Di Fusco D, Cacciotti L, Morganti R, D'Amato C, Greco C, Rufini S, Novelli G, Sangiuolo F, Spallone V, Borgiani P. MicroRNA genetic variations: association with type 2 diabetes. *Acta Diabetol* 2013; **50**: 867-872 [PMID: 23532299 DOI: 10.1007/s00592-013-0469-7]

183 **Buraczynska M**, Zukowski P, Wacinski P, Ksiazek K, Zaluska W. Polymorphism in microRNA-196a2 contributes to the risk of cardiovascular disease in type 2 diabetes patients. *J Diabetes Complications* 2014; **28**: 617-620 [PMID: 24972764 DOI: 10.1016/j.jdiacomp.2014.05.006]

184 **Li Q**, Chen L, Chen D, Wu X, Chen M. Influence of microRNA-related polymorphisms on clinical outcomes in coronary artery disease. *Am J Transl Res* 2015; **7**: 393-400 [PMID: 25901206]

185 **Guo J**, Liu Z, Gong R. Long noncoding RNA: an emerging player in diabetes and diabetic kidney disease. *Clin Sci (Lond)* 2019; **133**: 1321-1339 [PMID: 31221822 DOI: 10.1042/CS20190372]

186 **Chung AC**. microRNAs in Diabetic Kidney Disease. *Adv Exp Med Biol* 2015; **888**: 253-269 [PMID: 26663187 DOI: 10.1007/978-3-319-22671-2_13]

187 **Kriegel AJ**, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. *Physiol Genomics* 2012; **44**: 237-244 [PMID: 22214600 DOI: 10.1152/physiolgenomics.00141.2011]

188 **Howe EN**, Cochrane DR, Richer JK. The miR-200 and miR-221/222 microRNA families: opposing effects on epithelial identity. *J Mammary Gland Biol Neoplasia* 2012; **17**: 65-77 [PMID: 22350980 DOI: 10.1007/s10911-012-9244-6]

189 **Abdelsalam M**, Wahab AM, El Sayed Zaki M, Motawea M. MicroRNA-451 as an Early Predictor of Chronic Kidney Disease in Diabetic Nephropathy. *Int J Nephrol* 2020; **2020**: 8075376 [PMID: 32855824 DOI: 10.1155/2020/8075376]

190 **Fourdinier O**, Schepers E, Metzinger-Le Meuth V, Glorieux G, Liabeuf S, Verbeke F, Vanholder R, Brigant B, Pletinck A, Diouf M, Burtey S, Choukroun G, Massy ZA, Metzinger L; European Uremic Toxin Work Group-EUTox. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. *Sci Rep* 2019; **9**: 4477 [PMID: 30872798 DOI: 10.1038/s41598-019-41101-8]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 997-1009

DOI: 10.4239/wjd.v12.i7.997

ISSN 1948-9358 (online)

REVIEW

Mechanisms of altered bone remodeling in children with type 1 diabetes

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Author contributions: Faienza MF and Brunetti G equally contributed to this paper with conception and design of the article; Faienza MF drafted and revised the paper; D'Amato G and De Santis S performed literature review; Grano M critically revised the final version.

Conflict-of-interest statement: No potential conflicts of interest to declare.

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Abstract

Bone loss associated with type 1 diabetes mellitus (T1DM) begins at the onset of the disease, already in childhood, determining a lower bone mass peak and hence a greater risk of osteoporosis and fractures later in life. The mechanisms underlying diabetic bone fragility are not yet completely understood. Hyperglycemia and insulin deficiency can affect the bone cells functions, as well as the bone marrow fat, thus impairing the bone strength, geometry, and microarchitecture. Several factors, like insulin and growth hormone/insulin-like growth factor 1, can control bone marrow mesenchymal stem cell commitment, and the receptor activator of nuclear factor-κB ligand/osteoprotegerin and Wnt-b catenin pathways can impair bone turnover. Some myokines may have a key role in regulating metabolic control and improving bone mass in T1DM subjects. The aim of this review is to provide an overview of the current knowledge of the mechanisms underlying altered bone remodeling in children affected by T1DM.

Key Words: Type 1 diabetes; Children; Bone remodeling; Osteoclasts; Osteoblasts

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Italy

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 29, 2021
Peer-review started: January 29, 2021
First decision: March 1, 2021
Revised: March 17, 2021
Accepted: May 22, 2021
Article in press: May 22, 2021
Published online: July 15, 2021

P-Reviewer: Larkin J
S-Editor: Liu M
L-Editor: Filipodia
P-Editor: Liu JH

Core Tip: Bone fragility is a well-known complication related to type 1 diabetes mellitus, and it can manifest from the disease onset, already in childhood. The mechanisms underlying this relationship, and the precise role of metabolic control in preventing bone impairment, are not yet fully understood. Future studies are needed to clarify better the factors responsible for bone damage in diabetic subjects, and to identify strategies for avoiding and managing osteopenia/osteoporosis in these subjects.

Citation: Brunetti G, D'Amato G, De Santis S, Grano M, Faienza MF. Mechanisms of altered bone remodeling in children with type 1 diabetes. *World J Diabetes* 2021; 12(7): 997-1009
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/997.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.997

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a common endocrine disease that affects approximately 500000 children and adolescents worldwide. Moreover, in the last decade the incidence has been rising and the age at onset dropping[1]. Micro and macrovascular T1DM-related complications may already have developed a few years after disease onset[2] and are correlated with the age at onset, diabetes duration, body mass index, and pubertal development[3-5]. Hyperglycemia is the main systemic risk factor for diabetic complications, although multiple biochemical pathways, such as the formation of advanced glycation end products, oxidative stress, endoplasmic reticulum stress, inflammatory cytokines, and kallikrein-bradykinin activation, link the adverse effects of hyperglycemia with the microvascular dysfunction[6]. Consequently, long-term glycemic control is considered the most important modifiable factor to delay the onset, as well as the progression of microvascular complications, as clearly demonstrated by The Diabetes Control and Complications Trial[7]. In addition, beyond the effect of glycemic control, the impairment of some protective factors, such as insulin and insulin-like growth factor-1 (IGF1), may contribute to the vascular damage over time[8].

Recently, it was recognized that autoimmune diabetic disease also affects the skeleton[9-11]. In T1DM, a reduced bone mass may be present at an early stage after diagnosis[11], but it is unclear whether it is the duration of diabetes or degree of glycemic control that may induce a lifelong increased risk of fractures[12]. The association between glucose metabolism and bone-fat tissue interactions[13,14], as well as muscle-bone crosstalk, has been clearly demonstrated[15]. In particular, the skeleton acts as an endocrine organ, by modulating glucose tolerance through the secretion of bone-specific proteins, in particular osteocalcin (OCN). Furthermore, proteins involved in bone remodeling, like osteoprotegerin (OPG), are associated with an impaired insulin function[16].

The aim of this review is to provide an overview of current knowledge of the mechanisms underlying altered bone remodeling in children affected by T1DM.

FACTORS INFLUENCING BONE MASS ACCRUAL IN T1DM CHILDREN AND ADOLESCENTS

Childhood and adolescence are the critical ages for linear growth, bone mineral accrual, and the attainment of the peak bone mass, which is a key determinant of the lifelong risk of osteoporosis[17,18]. Therefore, osteoporosis prevention begins by improving bone mineral gains during an individual's years of growth[19]. During peripuberty, the bone mineral content and bone mineral density (BMD) in the lumbar spine and proximal femur increase by four-fold to six-fold. Furthermore, puberty is also the time when the main gender differences in bone growth emerge, particularly in terms of bone size and bone mass content. At the same time, the peak T1DM onset time ranges between the ages of 9 and 14 years[20], so children and adolescents affected by T1DM may be particularly predisposed to bone impairment.

Among the risk factors for osteoporosis, some factors are modifiable, such as a balanced diet and exercise, which have an important role already in childhood,

whereas obvious non-modifiable factors include gender, age, genetic factors, diseases, and drugs[21,22].

Impaired bone mass accrual (density and quality) in T1DM children has been attributed to multiple local factors in the bone marrow, as well as to systemic factors, which affect osteoblast (OB) differentiation and function (Figure 1).

Local factors in the bone marrow

Several studies have suggested that hyperglycemia impairs the biology and function of multipotent bone marrow-derived mesenchymal stem cells (BMSCs), which generate mesodermal tissues including cartilage, bone, muscle, tendon, ligament, and fat[23]. In particular, hyperglycemia both reduces the proliferation and increases the senescence of BMSCs *in vitro*[24,25] and also inhibits OB activities[26] (Figure 1). In addition, runt-related transcription factor 2 (*RUNX2*) and *RUNX2*-related osteogenic genes are downregulated in T1DM[27], suggesting that diabetic conditions may affect the BMSC fate commitment. Chronic hyperglycemia increases the expression of peroxisome proliferator-activated receptors (*PPAR*) genes, which also stimulate BMSCs differentiation in bone marrow adipocytes[28] (Figure 1). In addition to this, thiazolidinediones, antidiabetic PPARγ agonists, promote marrow adipogenesis, thus increasing the fracture risk[29]. The bone marrow adipose tissue (BMAT) is considered to be a single anatomical entity with a different distribution in the various skeletal sites. In an animal T1DM model, BMAT was significantly augmented, and bone formation was inversely associated with the adipocytes in the bone marrow[30]. BMAT also directly regulates osteoclastogenesis by producing receptor activator of nuclear factor-κB ligand (RANKL)[31]. Bone morphogenetic protein-6 (BMP6) is known to induce bone formation (Figure 1), and adipose-derived BMSCs overexpressing BMP6 have been shown to be capable of repairing bone defects in an animal model[32]. In addition, BMP6 can probably mitigate T1DM-associated bone loss by directing BMSC differentiation towards the osteogenic lineage. Recently, BMP6 supplementation in streptozotocin-induced diabetic mice has been demonstrated to directly restore BMD without influencing glucose levels[33], although a possible indirect role of BMP6 exerted through the modulation of glucose concentrations was observed[34].

This finding suggests that hyperglycemia may not be the main determinant of bone loss in T1DM patients, since other factors, like insulin and the growth hormone/insulin-like growth factor 1 (GH/IGF-1) axis, could modulate BMSCs osteogenesis *via* BMP6 or other pathways.

Insulin and GH/IGF-1 axis

Insulin, GH, and IGF-1 are anabolic hormones that directly affect bone cells (Figure 1). Insulin stimulates both osteoclast (OC) formation and OB proliferation, achieving a steady state in favor of bone formation[35]. Insulin signaling is essential for normal bone acquisition, as demonstrated in insulin receptor (IR) knockout mice[36], likely due to the role of insulin in the regulation of bone energy metabolism. Moreover, IR activation in the growth plate of mice fed with a hypercaloric diet stimulates skeletal growth as well as growth plate chondrogenesis[37]. OBs also express the IGF-1 receptor (IGF1R), and IGF-1 binds both to IGF1R and, with a lower affinity, to IR, thus triggering the insulin signaling pathway and exerting osteoanabolic activities.

Linear growth as well as BMD are critically affected by the GH/IGF-1 axis. Moreover, GH and IGF-1 play a key role at the growth plate, acting on chondrocyte proliferation, differentiation, and hypertrophy. Abnormalities in this axis have been reported in T1DM subjects, especially during puberty because of the increased insulin requirements due to physiological insulin resistance. In particular, T1DM patients exhibit GH hypersecretion, resulting from portal insulinopenia associated with a decreased hepatic output of IGF-1 together with pituitary hypersecretion of GH. The low IGF-1 serum levels are also related to increased levels of the inflammatory cytokines interleukin-6 and interleukin-8, which inhibit IGF-1 transcription[38] (Figure 1).

Several studies have established correlations between a low BMD, low IGF-1, and glycemic control in T1DM children and adolescents, also providing evidence of low BMD in subjects with poor glycemic control[39-42]. These data are correlated with reduced IGF1, IGF1R and transforming growth factor β1 gene expression in peripheral blood mononuclear cells in T1DM patients[43]. In addition, changes in the levels of the IGF-1 binding proteins that modulate IGF-1 bioactivity in serum and tissues have been observed in T1DM subjects[44].

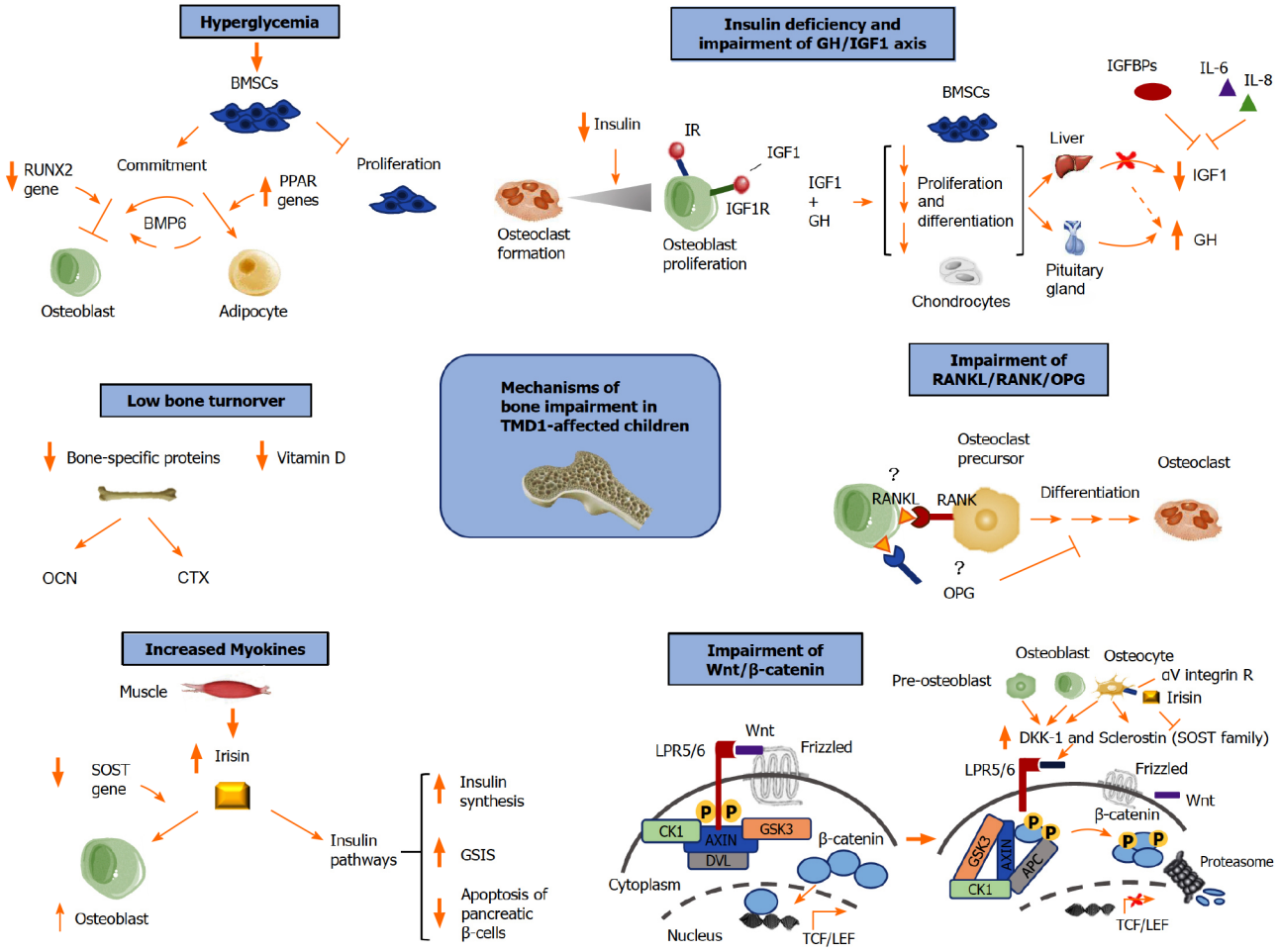

Figure 1 Mechanisms underlying altered bone remodeling in type-1-diabetes. APC: Adenomatous polyposis coli; BMP6: Bone morphogenetic protein-6; BMSCs: Bone marrow-derived mesenchymal stem cells; CK1: Casein kinase I; CTX: C-terminal cross-link of collagen; DKK-1: Dickkopf-1; DVL: Disheveled; GH: Growth hormone; GSIS: Glucose-stimulated insulin secretion; GSK3: Glycogen synthase kinase 3 beta; IR: Insulin receptor; IGF1: Insulin-like growth factor-1; IGF1R: Insulin-like growth factor-1 receptor; IGFBPs: IGF-1 binding proteins; IL-6: Interleukin-6; IL-8: Interleukin-8; LPR5/6: LDL receptor related protein 5; OCN: Osteocalcin; OPG: Osteoprotegerin; PPAR: Peroxisome proliferator-activated receptors; RANK: Receptor activator of nuclear factor-kappa B; RANKL: Receptor activator of nuclear factor-kappa B ligand; RUNX2: Related transcription factor 2; SOST: Sclerostin; TCF/LEF: T-cell factor/lymphoid enhancer factor.

RANKL/RANK/OPG pathway

Bone health depends on the balance between OCs, the bone-reabsorbing cells, and OBs, the bone-forming cells. In several pediatric diseases, bone impairment is due to an imbalance of OBs and OCs activity accomplishing the remodeling process[45]. OBs produce positive and negative regulators of osteoclastogenesis, such as the RANKL and the natural decoy receptor for RANKL, OPG, respectively[46]. Although OBs are a major source of RANKL, this cytokine is also expressed by osteocytes, fibroblasts, and immune system cells, including T cells and mature dendritic cells[47]. OCs differentiate under the control of RANKL, which binds to its receptor, RANK. OPG is the RANKL decoy receptor, thus acting as a negative regulator of osteoclastogenesis (Figure 1). OPG is produced not only by OBs but also by B lymphocytes and dendritic cells, as well as several cytokines[47,48]. In the last years, the impaired OB differentiation and function mechanisms in diabetic bone have been further elucidated, demonstrated by low serum levels of OB markers in T1DM subjects[49], and a decreased osteoblastic activity in streptozotocin-induced T1DM mice[50]. However, OC activity and bone resorption in T1DM are still debated. In diabetic animal models, an increase in OC numbers[51], as well as messenger RNA (mRNA levels of tartrate resistant acid phosphatase (TRAP) and cathepsin K, bone resorption markers, has been demonstrated[52,53]. By contrast, bone resorption was unaffected or even decreased in T1DM rodents[54]. In a recent study by Yang *et al*[55] the OC activity of trabecular bone was increased in diabetic mice at the early stage, accompanied by an augmented protein expression of RANKL. Remarkably, the RANKL mRNA levels remained unchanged, suggesting that the increased bone resorption in early-stage diabetic mice is induced by RANKL derived from BMAT rather than from the bone tissue itself[55].

This finding indicates that BMAT could be a key factor in regulating bone homeostasis in pathological conditions such as diabetes.

Data about OPG and RANKL levels in T1DM children and adolescents are conflicting. Chrysis *et al*[56] found that serum OPG levels were significantly increased in patients with T1DM compared with controls, whereas RANKL levels did not change. The low RANKL levels in T1DM patients are probably due to blockade of the RANKL signal by an OPG increase on the OPG/RANKL/RANK axis[56]. Consistently, Galluzzi *et al*[57] observed significantly higher levels of OPG in children with long-lasting T1DM compared to the controls. In the study by Szymańska *et al*[58], OPG levels were higher in T1DM subjects at the onset as compared to the control group, and decreased thereafter, while on the contrary, RANKL levels were lower than in controls but increased during follow-up. The authors speculated that the decreased insulin secretion in patients at the onset of diabetes may result in decreased insulin binding to the OB receptor, leading to a transitory increase of OPG levels in the early stage of diabetes[58]. Loureiro *et al*[59] reported low OPG levels in T1DM children, which were correlated with the metabolic control level. In the recent study by Karalazou *et al*[60], T1DM patients showed higher RANKL levels and lower OPG levels than controls. Taking literature data into account, high OPG levels would seem to be positively associated with the progression of diabetes and the development of complications[61-63], while low OPG levels are not associated with microvascular alterations[64,65].

Wnt/β-catenin pathway

The Wnt/β-catenin pathway is a signal transduction cascade that controls numerous processes during development. Thus, aberrant Wnt signaling underlies a broad range of diseases in humans.

The pathway is regulated at several levels, also by secreted Frizzled-related proteins and Wnt inhibitory protein, both of which inhibit interactions between Wnt and Wnt receptors[66] (Figure 1). Other Wnt inhibitors belong to the Dickkopf-1 (DKK-1) and the WISE/SOST families, which antagonize signaling by binding low-density lipoprotein-related receptor-5/6[67]. DKK-1 is expressed by preosteoblasts, OBs, and osteocytes, and acts as an antagonist of the canonical Wnt signaling by binding to low-density lipoprotein-related receptor-5/6 (Figure 1). Sclerostin is a secreted protein encoded by the *SOST* gene and produced by mature osteocytes, which antagonizes Wnt/β-catenin signaling by abrogating its bone anabolic actions[68,69] (Figure 1). DKK-1 and sclerostin are key regulators of bone mass, and high levels of these Wnt signaling inhibitors have been found in several bone diseases[70]. The important role of both molecules has also been demonstrated in a mouse model, showing that a bispecific antibody targeting sclerostin and DKK-1 supports bone mass accrual and fracture repair, exerting a greater effect compared to monotherapies[71]. In type 1 diabetic rats, an increased *SOST* mRNA and sclerostin expression has been observed [72]. Clinical studies and Homeostatic Model Assessment for Insulin Resistance have shown an inverse correlation between sclerostin and insulin levels, suggesting that sclerostin could modulate glucose homeostasis[73]. DKK-1 involvement has been demonstrated in a large cohort of T1DM children and adolescents affected by T1DM [74], in which DKK-1 levels were correlated with bone formation markers, the BMD-Z-score, sex, and pubertal stage[74]. Neumann *et al*[75] reported higher serum levels of sclerostin in T1DM subjects compared with controls but found no correlations between sclerostin levels and bone metabolism markers. On the contrary, Tsentidis *et al*[76] found comparable levels of sclerostin in T1DM children and controls. Recent data suggested that sclerostin levels are increased in pediatric T1MD patients and confirmed a relationship between sclerostin and the glucose metabolism[77]. In addition, in T1MD subjects' bone-derived OCN, as well as fat-derived leptin, appear to modulate sclerostin support in metabolic regulation[77]. Future studies are needed to clarify the role of sclerostin in bone impairment associated with T1DM.

Muscle-bone crosstalk

Bone and muscles are integrated organs that exert a mutual control and are in turn controlled by several factors, such as the GH-IGF-1 axis, sex steroids, adipokines (*e.g.*, leptin, adiponectin, visfatin, resistin), and vitamin D[78,79]. In addition to mediating the muscle-bone crosstalk, muscles release myokines that affect other organs and tissues, including the liver, intestine, and adipose tissue, which in turn release cytokines and hormones responsible for regulating bone homeostasis. Among the myokines, irisin is a small peptide derived from the proteolytic cleavage of fibronectin III domain-containing protein 5, produced during physical exercise[80]. This myokine has been associated with the browning response and thermogenesis of white adipose

tissue[80]. In addition, it has an essential role in the bone-muscle unit, and exerts anabolic effects on bone, both *in vitro* and *in vivo*[81]. Irisin acts through the activation of osteoblastic bone formation, the induction of the pro-osteoblastic genes, and the decrease of osteoblastogenesis inhibitors[81]. The direct effect of irisin on OBs is exerted by means of a downregulation of *SOST* expression, which negatively regulates bone formation[81] (Figure 1). In agreement with this finding, Zhang *et al*[82] demonstrated *in vitro* that treatment with recombinant irisin on OB precursors causes the accumulation of β-catenin in the nucleus, suggesting that irisin restores SOST-mediated inhibition of the Wnt/β-catenin pathway by directly inhibiting *SOST*. In addition, irisin interacts with osteocytes by directly binding to αV integrin receptors, thus protecting them from apoptosis, and inducing the secretion of *SOST in vivo*[83]. Regarding its metabolic effects, recombinant irisin has been shown to stimulate insulin biosynthesis and glucose-stimulated insulin secretion in a protein kinase A-dependent manner. It also prevents saturated fatty acid-induced apoptosis in human and rat pancreatic β cells, as well as in human and murine pancreatic islets, *via* the AKT/B-cell lymphoma 2 signaling pathway[84] (Figure 1). Studies in humans have elucidated the role of irisin both in healthy subjects and in patients affected by diseases related to bone metabolism, such as hyperparathyroidism and T1DM. In a recent study, irisin was demonstrated to be one of the main determinants of bone mineral status during childhood[85]. In addition, high irisin levels have been found in adult patients with long-lasting T1DM that were correlated with positivity for anti-glutamic acid decarboxylase antibodies, suggesting that autoimmunity can have a role in regulating the levels of this myokine[86,87].

In T1DM children and adolescents, elevated irisin levels have been found to be closely related to better metabolic control and an improved bone mass[88]. These findings are in agreement with the recent data showing that irisin can promote insulin synthesis as well as glucose-stimulated insulin secretion[84]. In addition, irisin overexpression enhanced insulin sensitivity in mice while reducing hyperlipidemia and hyperglycemia[89], suggesting that irisin could have a key role in diabetes management.

BONE TURNOVER MARKERS IN T1DM CHILDREN

Bone is considered to be an endocrine "gland," and its modulation of glucose tolerance by the secretion of bone-specific proteins, in particular OCN, has been clearly demonstrated[16]. OCN is the main non-collagen protein secreted by the OBs and stored in the bone extracellular matrix. The carboxylated form of OCN shows a high affinity to hydroxyapatite, the mineral present in bone. Instead, the uncarboxylated form is free in the circulation and regulates glucose metabolism and insulin resistance [90]. Several data have suggested that serum levels of uncarboxylated OCN are negatively correlated with insulin resistance, obesity, diabetes, and markers of the metabolic syndrome[91-93].

T1DM subjects show a low bone turnover, which is another mechanism underlying bone fragility in these subjects[94]. Previous studies demonstrated that both markers of bone resorption, such as the C-terminal cross-link of collagen (CTX), and markers of bone formation, such as OCN, were decreased in T1DM compared to healthy controls [94]. Furthermore, while levels of TRAP and procollagen type 1 amino terminal propeptide (P1NP) were comparable in patients with T1DM and healthy subjects, low vitamin D levels were found in T1DM patients[94]. Similarly, reduced OCN levels were observed in children and adolescents with T1DM, while P1NP levels would seem to be lower and CTX levels higher in T1DM than in healthy subjects[95]. Chen *et al*[96] showed low levels of bone alkaline phosphatase and CTX in T1DM children as compared to controls.

A recent report by Madsen *et al*[97] investigated bone turnover markers in relation to BMD and metabolic control in T1DM children and adolescents. The results of this study demonstrated that markers of bone formation and resorption were significantly decreased in both sexes, and HbA1c levels were negatively correlated to the resorption marker CTX but not to any of the bone formation markers[97]. Another important finding of this study was that the decreased levels of both markers of bone formation and bone resorption were independent of the T1DM duration and Tanner stage. Thus, the impairment of bone health in T1DM begins in early childhood, independently of age and pubertal stage.

A possible explanation for the low bone turnover in diabetic subjects may be an insulin deficiency, which contributes to a low bone formation, as demonstrated by low

bone turnover in a mouse model of insulinopenia and restoration following insulin treatment[98].

It is possible that the low bone turnover caused by insulin deficiency occurs over time and may not be detected in studies based on acute changes in insulin levels[99].

FUTURE PROSPECTS IN THE MANAGEMENT OF BONE IMPAIRMENT IN T1DM SUBJECTS

T1DM is the most frequent chronic disease in the pediatric population. It is associated with an increased bone fragility from childhood onward, and hence the risk of fractures later in life. Although a low BMD is documented in diabetic individuals, the precise mechanisms underlying bone loss are not yet fully understood. Hyperglycemia seems not to be the main cause of bone impairment in T1DM patients, but other factors, like insulin deficiency, the GH/IGF-1 axis, and low bone turnover, could contribute to the bone impairment observed in these subjects. The use of diabetes technologies, like the use of insulin pumps and continuous glucose monitors, to achieve glycemia control appears to be correlated with an improved bone health, although further studies are needed to confirm this finding. In addition, prospective studies should clarify the causal relationships among metabolic control, bone turnover markers, the RANKL/OPG ratio, Wnt-signaling inhibitors, myokine activity, and bone mineralization in T1DM subjects.

To date, no specific biomarkers are available to predict accurately fracture outcomes in T1DM patients. Additional large-scale prospective studies are needed to identify high-risk patients. In addition to dual x-ray absorptiometry, a fracture risk assessment tool and trabecular bone score could, in the future, offer additional technologies to evaluate better the bone quality of T1DM patients[100].

There is no clear evidence in support of early intervention to avoid the risk of osteoporosis or of the use of anti-osteoporotic drugs in diabetic subjects[101].

Preclinical studies indicated that denosumab, a human monoclonal antibody to RANKL approved for the treatment of osteoporosis or for patients at high fracture risk [102], may stimulate β-cell proliferation in humans[103] and improve liver insulin sensitivity[104].

No data are currently available on romosozumab, an anti-sclerostin antibody indicated to reduce the risk of clinical and vertebral fractures in postmenopausal women with osteoporosis[105]. In addition, there are still few data on the effects of vitamin D, calcium intake, and physical activity on bone health in T1DM subjects [106]. Recently, toll-like receptor-4 (TLR4) has been correlated with diabetic bone disorders *via* the nuclear factor-κB pathway[107,108]. It has been demonstrated that TLR4 deletion improves streptozotocin-induced diabetic osteoporosis in mice, so TLR4 may be a possible therapeutic target for the treatment of diabetic osteoporosis[109].

CONCLUSION

T1DM has a strong impact on bone health, and skeletal fragility is now recognized among the complications of diabetes. The fracture risk is greater in patients with T1DM and increases linearly with the disease duration. T1DM subjects show a decreased BMD already in childhood, possibly due to an absolute insulin deficiency and the inability of exogenous insulin to reflect endogenous insulin secretion. However, the reduction in BMD does not entirely explain the increase in bone fragility observed in these subjects. It is unclear whether reducing hypoglycemic events by means of continuous glucose monitoring and a closed-loop insulin delivery system can improve bone health in subjects with T1DM. Randomized clinical trials to evaluate the efficacy of anti-fracture drugs in diabetes are lacking, while some observational data have indicated an analogous efficacy in those with or without diabetes, so such drugs should be used according to existing indications.

Further studies are warranted to clarify better the factors responsible for bone damage in diabetic subjects and to identify efficacious strategies to prevent osteopenia/osteoporosis and the risk of fractures in these subjects.

REFERENCES

1 **Ogurtsova K**, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017; **128**: 40-50 [PMID: 28437734 DOI: 10.1016/j.diabres.2017.03.024]

2 **Donaghue KC**, Wadwa RP, Dimeglio LA, Wong TY, Chiarelli F, Marcovecchio ML, Salem M, Raza J, Hofman PL, Craig ME; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. Microvascular and macrovascular complications in children and adolescents. *Pediatr Diabetes* 2014; **15** Suppl 20: 257-269 [PMID: 25182318 DOI: 10.1111/pedi.12180]

3 **Faienza MF**, Acquafredda A, Tesse R, Luce V, Ventura A, Maggialetti N, Monteduro M, Giordano P, Cavallo L. Risk factors for subclinical atherosclerosis in diabetic and obese children. *Int J Med Sci* 2013; **10**: 338-343 [PMID: 23423872 DOI: 10.7150/ijms.5181]

4 **Demirel F**, Tepe D, Kara O, Esen I. Microvascular complications in adolescents with type 1 diabetes mellitus. *J Clin Res Pediatr Endocrinol* 2013; **5**: 145-149 [PMID: 24072081 DOI: 10.4274/Jcrpe.994]

5 **Svensson M**, Eriksson JW, Dahlquist G. Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: a population-based study in northern Sweden. *Diabetes Care* 2004; **27**: 955-962 [PMID: 15047655 DOI: 10.2337/diacare.27.4.955]

6 **Barrett EJ**, Liu Z, Khamaisi M, King GL, Klein R, Klein BEK, Hughes TM, Craft S, Freedman BI, Bowden DW, Vinik AI, Casellini CM. Diabetic Microvascular Disease: An Endocrine Society Scientific Statement. *J Clin Endocrinol Metab* 2017; **102**: 4343-4410 [PMID: 29126250 DOI: 10.1210/jc.2017-01922]

7 **Diabetes Control and Complications Trial Research Group**, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]

8 **Sun JK**, Keenan HA, Cavallerano JD, Asztalos BF, Schaefer EJ, Sell DR, Strauch CM, Monnier VM, Doria A, Aiello LP, King GL. Protection from retinopathy and other complications in patients with type 1 diabetes of extreme duration: the joslin 50-year medalist study. *Diabetes Care* 2011; **34**: 968-974 [PMID: 21447665 DOI: 10.2337/dc10-1675]

9 **Shah VN**, Shah CS, Snell-Bergeon JK. Type 1 diabetes and risk of fracture: meta-analysis and review of the literature. *Diabet Med* 2015; **32**: 1134-1142 [PMID: 26096918 DOI: 10.1111/dme.12734]

10 **Hough FS**, Pierroz DD, Cooper C, Ferrari SL; IOF CSA Bone and Diabetes Working Group. MECHANISMS IN ENDOCRINOLOGY: Mechanisms and evaluation of bone fragility in type 1 diabetes mellitus. *Eur J Endocrinol* 2016; **174**: R127-R138 [PMID: 26537861 DOI: 10.1530/EJE-15-0820]

11 **Weber DR**, Schwartz G. Epidemiology of Skeletal Health in Type 1 Diabetes. *Curr Osteoporos Rep* 2016; **14**: 327-336 [PMID: 27744554 DOI: 10.1007/s11914-016-0333-0]

12 **Napoli N**, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL; IOF Bone and Diabetes Working Group. Mechanisms of diabetes mellitus-induced bone fragility. *Nat Rev Endocrinol* 2017; **13**: 208-219 [PMID: 27658727 DOI: 10.1038/nrendo.2016.153]

13 **Lecka-Czernik B**, Rosen CJ. Energy Excess, Glucose Utilization, and Skeletal Remodeling: New Insights. *J Bone Miner Res* 2015; **30**: 1356-1361 [PMID: 26094610 DOI: 10.1002/jbmr.2574]

14 **Abdalrahaman N**, McComb C, Foster JE, Lindsay RS, Drummond R, McKay GA, Perry CG, Ahmed SF. The relationship between adiposity, bone density and microarchitecture is maintained in young women irrespective of diabetes status. *Clin Endocrinol (Oxf)* 2017; **87**: 327-335 [PMID: 28656591 DOI: 10.1111/cen.13410]

15 **Colaianni G**, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, Lu P, Sartini L, Di Comite M, Mori G, Di Benedetto A, Brunetti G, Yuen T, Sun L, Reseland JE, Colucci S, New MI, Zaidi M, Cinti S, Grano M. The myokine irisin increases cortical bone mass. *Proc Natl Acad Sci USA* 2015; **112**: 12157-12162 [PMID: 26374841 DOI: 10.1073/pnas.1516622112]

16 **Faienza MF**, Luce V, Ventura A, Colaianni G, Colucci S, Cavallo L, Grano M, Brunetti G. Skeleton and glucose metabolism: a bone-pancreas loop. *Int J Endocrinol* 2015; **2015**: 758148 [PMID: 25873957 DOI: 10.1155/2015/758148]

17 **Sopher AB**, Fennoy I, Oberfield SE. An update on childhood bone health: mineral accrual, assessment and treatment. *Curr Opin Endocrinol Diabetes Obes* 2015; **22**: 35-40 [PMID: 25517023 DOI: 10.1097/MED.0000000000000124]

18 **Weaver CM**, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, O'Karma M, Wallace TC, Zemel BS. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int* 2016; **27**: 1281-1386 [PMID: 26856587 DOI: 10.1007/s00198-015-3440-3]

19 **Carey DE**, Golden NH. Bone Health in Adolescence. *Adolesc Med State Art Rev* 2015; **26**: 291-325 [PMID: 26999874]

20 **Royal College of Paediatrics and Child Health**. National Paediatric Diabetes Audit (NPDA) national reports; 2016 [cited 10 January 2021]. [Internet]. Available from:

https://www.rcpch.ac.uk/sites/default/files/2018-03/npda_national_report_2013-14.pdf

21 **Stefania S**, Clodoveo ML, Cariello M, D'Amato G, Franchini C, Faienza MF, Corbo F. Polyphenols and obesity prevention: critical insights on molecular regulation, bioavailability and dose in preclinical and clinical settings. *Crit Rev Food Sci Nutr* 2020; 1-23 [PMID: 32436425 DOI: 10.1080/10408398.2020.1765736]

22 **Faienza MF**, Lassandro G, Chiarito M, Valente F, Ciaccia L, Giordano P. How Physical Activity across the Lifespan Can Reduce the Impact of Bone Ageing: A Literature Review. *Int J Environ Res Public Health* 2020; **17**: 1862 [PMID: 32183049 DOI: 10.3390/ijerph17061862]

23 **Oreffo RO**, Cooper C, Mason C, Clements M. Mesenchymal stem cells: lineage, plasticity, and skeletal therapeutic potential. *Stem Cell Rev* 2005; **1**: 169-178 [PMID: 17142852 DOI: 10.1385/SCR:1:2:169]

24 **Chang TC**, Hsu MF, Wu KK. High glucose induces bone marrow-derived mesenchymal stem cell senescence by upregulating autophagy. *PLoS One* 2015; **10**: e0126537 [PMID: 25961745 DOI: 10.1371/journal.pone.0126537]

25 **Li YM**, Schilling T, Benisch P, Zeck S, Meissner-Weigl J, Schneider D, Limbert C, Seufert J, Kassem M, Schütze N, Jakob F, Ebert R. Effects of high glucose on mesenchymal stem cell proliferation and differentiation. *Biochem Biophys Res Commun* 2007; **363**: 209-215 [PMID: 17868648 DOI: 10.1016/j.bbrc.2007.08.161]

26 **Cao B**, Liu N, Wang W. High glucose prevents osteogenic differentiation of mesenchymal stem cells *via* lncRNA AK028326/CXCL13 pathway. *Biomed Pharmacother* 2016; **84**: 544-551 [PMID: 27693963 DOI: 10.1016/j.biopha.2016.09.058]

27 **Fowlkes JL**, Bunn RC, Liu L, Wahl EC, Coleman HN, Cockrell GE, Perrien DS, Lumpkin CK Jr, Thrailkill KM. Runt-related transcription factor 2 (RUNX2) and RUNX2-related osteogenic genes are down-regulated throughout osteogenesis in type 1 diabetes mellitus. *Endocrinology* 2008; **149**: 1697-1704 [PMID: 18162513 DOI: 10.1210/en.2007-1408]

28 **Botolin S**, McCabe LR. Chronic hyperglycemia modulates osteoblast gene expression through osmotic and non-osmotic pathways. *J Cell Biochem* 2006; **99**: 411-424 [PMID: 16619259 DOI: 10.1002/jcb.20842]

29 **Kim TY**, Schafer AL. Diabetes and Bone Marrow Adiposity. *Curr Osteoporos Rep* 2016; **14**: 337-344 [PMID: 27714580 DOI: 10.1007/s11914-016-0336-x]

30 **Motyl KJ**, Raetz M, Tekalur SA, Schwartz RC, McCabe LR. CCAAT/enhancer binding protein β-deficiency enhances type 1 diabetic bone phenotype by increasing marrow adiposity and bone resorption. *Am J Physiol Regul Integr Comp Physiol* 2011; **300**: R1250-R1260 [PMID: 21346244 DOI: 10.1152/ajpregu.00764.2010]

31 **Fan Y**, Hanai JI, Le PT, Bi R, Maridas D, DeMambro V, Figueroa CA, Kir S, Zhou X, Mannstadt M, Baron R, Bronson RT, Horowitz MC, Wu JY, Bilezikian JP, Dempster DW, Rosen CJ, Lanske B. Parathyroid Hormone Directs Bone Marrow Mesenchymal Cell Fate. *Cell Metab* 2017; **25**: 661-672 [PMID: 28162969 DOI: 10.1016/j.cmet.2017.01.001]

32 **Sheyn D**, Tawackoli W, Gazit Z, Pelled G. Allogeneic, BMP6 gene-modified, bone marrow MSCs induce vertebral fracture repair ina porcine model: A pilot study. *Spine J* 2013; **13**: S71-S72 [DOI: 10.1016/j.spinee.2013.07.200]

33 **Wang JF**, Lee MS, Tsai TL, Leiferman EM, Trask DJ, Squire MW, Li WJ. Bone Morphogenetic Protein-6 Attenuates Type 1 Diabetes Mellitus-Associated Bone Loss. *Stem Cells Transl Med* 2019; **8**: 522-534 [PMID: 30784225 DOI: 10.1002/sctm.18-0150]

34 **Vukicevic S**, Grgurevic L. BMP-6 and mesenchymal stem cell differentiation. *Cytokine Growth Factor Rev* 2009; **20**: 441-448 [PMID: 19900832 DOI: 10.1016/j.cytogfr.2009.10.020]

35 **Pramojanee SN**, Phimphilai M, Chattipakorn N, Chattipakorn SC. Possible roles of insulin signaling in osteoblasts. *Endocr Res* 2014; **39**: 144-151 [PMID: 24679227 DOI: 10.3109/07435800.2013.879168]

36 **Fulzele K**, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, Faugere MC, Aja S, Hussain MA, Brüning JC, Clemens TL. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell* 2010; **142**: 309-319 [PMID: 20655471 DOI: 10.1016/j.cell.2010.06.002]

37 **Wu S**, Zhang Y, De Luca F. The effect of a high-calorie diet on bone growth is mediated by the insulin receptor. *Bone* 2019; **122**: 166-175 [PMID: 30798001 DOI: 10.1016/j.bone.2019.02.021]

38 **Van Sickle BJ**, Simmons J, Hall R, Raines M, Ness K, Spagnoli A. Increased circulating IL-8 is associated with reduced IGF-1 and related to poor metabolic control in adolescents with type 1 diabetes mellitus. *Cytokine* 2009; **48**: 290-294 [PMID: 19773182 DOI: 10.1016/j.cyto.2009.08.011]

39 **Heap J**, Murray MA, Miller SC, Jalili T, Moyer-Mileur LJ. Alterations in bone characteristics associated with glycemic control in adolescents with type 1 diabetes mellitus. *J Pediatr* 2004; **144**: 56-62 [PMID: 14722519 DOI: 10.1016/j.jpeds.2003.10.066]

40 **de Souza KS**, Ururahy MA, da Costa Oliveira YM, Loureiro MB, da Silva HP, Bortolin RH, Melo Dos Santos F, Luchessi AD, Neto JJ, Arrais RF, Hirata RD, das Graças Almeida M, Hirata MH, de Rezende AA. Low bone mineral density in patients with type 1 diabetes: association with reduced expression of IGF1, IGF1R and TGF B 1 in peripheral blood mononuclear cells. *Diabetes Metab Res Rev* 2016; **32**: 589-595 [PMID: 26663878 DOI: 10.1002/dmrr.2772]

41 **Valerio G**, del Puente A, Esposito-del Puente A, Buono P, Mozzillo E, Franzese A. The lumbar bone mineral density is affected by long-term poor metabolic control in adolescents with type 1 diabetes mellitus. *Horm Res* 2002; **58**: 266-272 [PMID: 12446989 DOI: 10.1159/000066441]

42 **Moyer-Mileur LJ**, Dixon SB, Quick JL, Askew EW, Murray MA. Bone mineral acquisition in adolescents with type 1 diabetes. *J Pediatr* 2004; **145**: 662-669 [PMID: 15520770 DOI: 10.1016/j.jpeds.2004.06.070]

43 **Raisingani M**, Preneet B, Kohn B, Yakar S. Skeletal growth and bone mineral acquisition in type 1 diabetic children; abnormalities of the GH/IGF-1 axis. *Growth Horm IGF Res* 2017; **34**: 13-21 [PMID: 28482269 DOI: 10.1016/j.ghir.2017.04.003]

44 **Sharma A**, Purohit S, Sharma S, Bai S, Zhi W, Ponny SR, Hopkins D, Steed L, Bode B, Anderson SW, She JX. IGF-Binding Proteins in Type-1 Diabetes Are More Severely Altered in the Presence of Complications. *Front Endocrinol (Lausanne)* 2016; **7**: 2 [PMID: 26858687 DOI: 10.3389/fendo.2016.00002]

45 **Brunetti G**, D'Amato G, Chiarito M, Tullo A, Colaianni G, Colucci S, Grano M, Faienza MF. An update on the role of RANKL-RANK/osteoprotegerin and WNT-ß-catenin signaling pathways in pediatric diseases. *World J Pediatr* 2019; **15**: 4-11 [PMID: 30343446 DOI: 10.1007/s12519-018-0198-7]

46 **Rauner M**, Sipos W, Pietschmann P. Osteoimmunology. *Int Arch Allergy Immunol* 2007; **143**: 31-48 [PMID: 17191007 DOI: 10.1159/000098223]

47 **Brunetti G**, Di Benedetto A, Posa F, Colaianni G, Faienza MF, Ballini A, Colucci S, Passeri G, Lo Muzio L, Grano M, Mori G. High expression of TRAIL by osteoblastic differentiated dental pulp stem cells affects myeloma cell viability. *Oncol Rep* 2018; **39**: 2031-2039 [PMID: 29484421 DOI: 10.3892/or.2018.6272]

48 **Soysa NS**, Alles N, Aoki K, Ohya K. Osteoclast formation and differentiation: an overview. *J Med Dent Sci* 2012; **59**: 65-74 [PMID: 23897045]

49 **Tsentidis C**, Gourgiotis D, Kossiva L, Doulgeraki A, Marmarinos A, Galli-Tsinopoulou A, Karavanaki K. Higher levels of s-RANKL and osteoprotegerin in children and adolescents with type 1 diabetes mellitus may indicate increased osteoclast signaling and predisposition to lower bone mass: a multivariate cross-sectional analysis. *Osteoporos Int* 2016; **27**: 1631-1643 [PMID: 26588909 DOI: 10.1007/s00198-015-3422-5]

50 **Peng J**, Hui K, Hao C, Peng Z, Gao QX, Jin Q, Lei G, Min J, Qi Z, Bo C, Dong QN, Bing ZH, Jia XY, Fu DL. Low bone turnover and reduced angiogenesis in streptozotocin-induced osteoporotic mice. *Connect Tissue Res* 2016; **57**: 277-289 [PMID: 27028715 DOI: 10.3109/03008207.2016.1171858]

51 **Alharbi MA**, Zhang C, Lu C, Milovanova TN, Yi L, Ryu JD, Jiao H, Dong G, O'Connor JP, Graves DT. FOXO1 Deletion Reverses the Effect of Diabetic-Induced Impaired Fracture Healing. *Diabetes* 2018; **67**: 2682-2694 [PMID: 30279162 DOI: 10.2337/db18-0340]

52 **Hie M**, Tsukamoto I. Increased expression of the receptor for activation of NF-kappaB and decreased runt-related transcription factor 2 expression in bone of rats with streptozotocin-induced diabetes. *Int J Mol Med* 2010; **26**: 611-618 [PMID: 20818503 DOI: 10.3892/ijmm_00000506]

53 **Hie M**, Shimono M, Fujii K, Tsukamoto I. Increased cathepsin K and tartrate-resistant acid phosphatase expression in bone of streptozotocin-induced diabetic rats. *Bone* 2007; **41**: 1045-1050 [PMID: 17916452 DOI: 10.1016/j.bone.2007.08.030]

54 **Botolin S**, Faugere MC, Malluche H, Orth M, Meyer R, McCabe LR. Increased bone adiposity and peroxisomal proliferator-activated receptor-gamma2 expression in type I diabetic mice. *Endocrinology* 2005; **146**: 3622-3631 [PMID: 15905321 DOI: 10.1210/en.2004-1677]

55 **Yang J**, Chen S, Zong Z, Yang L, Liu D, Bao Q, Du W. The increase in bone resorption in early-stage type I diabetic mice is induced by RANKL secreted by increased bone marrow adipocytes. *Biochem Biophys Res Commun* 2020; **525**: 433-439 [PMID: 32102755 DOI: 10.1016/j.bbrc.2020.02.079]

56 **Chrysis D**, Efthymiadou A, Mermigka A, Kritikou D, Spiliotis BE. Osteoprotegerin, RANKL, ADMA, and Fetuin-A serum levels in children with type I diabetes mellitus. *Pediatr Diabetes* 2017; **18**: 277-282 [PMID: 27028343 DOI: 10.1111/pedi.12384]

57 **Galluzzi F**, Stagi S, Salti R, Toni S, Piscitelli E, Simonini G, Falcini F, Chiarelli F. Osteoprotegerin serum levels in children with type 1 diabetes: a potential modulating role in bone status. *Eur J Endocrinol* 2005; **153**: 879-885 [PMID: 16322394 DOI: 10.1530/eje.1.02052]

58 **Szymańska M**, Michałus I, Kaszkowiak M, Wyka K, Chlebna-Sokół D, Fendler W, Jakubowska-Pietkiewicz E, Młynarski W, Szadkowska A, Zmysłowska A. Metabolic bone markers can be related to preserved insulin secretion in children with newly diagnosed type 1 diabetes. *Pediatr Endocrinol Diabetes Metab* 2020; **26**: 10-16 [PMID: 32418417 DOI: 10.5114/pedm.2020.94391]

59 **Loureiro MB**, Ururahy MA, Freire-Neto FP, Oliveira GH, Duarte VM, Luchessi AD, Brandão-Neto J, Hirata RD, Hirata MH, Maciel-Neto JJ, Arrais RF, Almeida MG, Rezende AA. Low bone mineral density is associated to poor glycemic control and increased OPG expression in children and adolescents with type 1 diabetes. *Diabetes Res Clin Pract* 2014; **103**: 452-457 [PMID: 24529565 DOI: 10.1016/j.diabres.2013.12.018]

60 **Karalazou P**, Ntelios D, Chatzopoulou F, Fragou A, Taousani M, Mouzaki K, Galli-Tsinopoulou A, Kouidou S, Tzimagiorgis G. OPG/RANK/RANKL signaling axis in patients with type I diabetes: Associations with parathormone and vitamin D. *Ital J Pediatr* 2019; **45**: 161 [PMID: 31823791 DOI: 10.1186/s13052-019-0748-1]

61 **Lambrinoudaki I**, Tsouvalas E, Vakaki M, Kaparos G, Stamatelopoulos K, Augoulea A, Pliatsika P, Alexandrou A, Creatsa M, Karavanaki K. Osteoprotegerin, Soluble Receptor Activator of Nuclear Factor- κ B Ligand, and Subclinical Atherosclerosis in Children and Adolescents with Type 1

Diabetes Mellitus. *Int J Endocrinol* 2013; **2013**: 102120 [PMID: 24288529 DOI: 10.1155/2013/102120]

62 **Venuraju SM**, Yerramasu A, Corder R, Lahiri A. Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *J Am Coll Cardiol* 2010; **55**: 2049-2061 [PMID: 20447527 DOI: 10.1016/j.jacc.2010.03.013]

63 **Wang ST**, Xu JM, Wang M, Chen FL, Ding G. Increased plasma osteoprotegerin concentrations in Type 1 diabetes with albuminuria. *Clin Nephrol* 2013; **79**: 192-198 [PMID: 23073066 DOI: 10.5414/CN107536]

64 **Singh DK**, Winocour P, Summerhayes B, Viljoen A, Sivakumar G, Farrington K. Low serum osteoprotegerin levels in normoalbuminuric type 1 diabetes mellitus. *Acta Diabetol* 2010; **47** Suppl 1: 105-110 [PMID: 19784545 DOI: 10.1007/s00592-009-0146-z]

65 **Abd El Dayem SM**, El-Shehaby AM, Abd El Gafar A, Fawzy A, Salama H. Bone density, body composition, and markers of bone remodeling in type 1 diabetic patients. *Scand J Clin Lab Invest* 2011; **71**: 387-393 [PMID: 21476826 DOI: 10.3109/00365513.2011.573574]

66 **Clevers H**, Nusse R. Wnt/β-catenin signaling and disease. *Cell* 2012; **149**: 1192-1205 [PMID: 22682243 DOI: 10.1016/j.cell.2012.05.012]

67 **Glinka A**, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 1998; **391**: 357-362 [PMID: 9450748 DOI: 10.1038/34848]

68 **Balemans W**, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuyts W, Van Den Ende J, Willems P, Paes-Alves AF, Hill S, Bueno M, Ramos FJ, Tacconi P, Dikkers FG, Stratakis C, Lindpaintner K, Vickery B, Foernzler D, Van Hul W. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum Mol Genet* 2001; **10**: 537-543 [PMID: 11181578 DOI: 10.1093/hmg/10.5.537]

69 **Plotkin LI**, Bellido T. Osteocytic signalling pathways as therapeutic targets for bone fragility. *Nat Rev Endocrinol* 2016; **12**: 593-605 [PMID: 27230951 DOI: 10.1038/nrendo.2016.71]

70 **Baron R**, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. *Nat Med* 2013; **19**: 179-192 [PMID: 23389618 DOI: 10.1038/nm.3074]

71 **Florio M**, Gunasekaran K, Stolina M, Li X, Liu L, Tipton B, Salimi-Moosavi H, Asuncion FJ, Li C, Sun B, Tan HL, Zhang L, Han CY, Case R, Duguay AN, Grisanti M, Stevens J, Pretorius JK, Pacheco E, Jones H, Chen Q, Soriano BD, Wen J, Heron B, Jacobsen FW, Brisan E, Richards WG, Ke HZ, Ominsky MS. A bispecific antibody targeting sclerostin and DKK-1 promotes bone mass accrual and fracture repair. *Nat Commun* 2016; **7**: 11505 [PMID: 27230681 DOI: 10.1038/ncomms11505]

72 **Pacicca DM**, Brown T, Watkins D, Kover K, Yan Y, Prideaux M, Bonewald L. Elevated glucose acts directly on osteocytes to increase sclerostin expression in diabetes. *Sci Rep* 2019; **9**: 17353 [PMID: 31757981 DOI: 10.1038/s41598-019-52224-3]

73 **Cipriani C**, Colangelo L, Santori R, Renella M, Mastrantonio M, Minisola S, Pepe J. The Interplay Between Bone and Glucose Metabolism. *Front Endocrinol (Lausanne)* 2020; **11**: 122 [PMID: 32265831 DOI: 10.3389/fendo.2020.00122]

74 **Faienza MF**, Ventura A, Delvecchio M, Fusillo A, Piacente L, Aceto G, Colaianni G, Colucci S, Cavallo L, Grano M, Brunetti G. High Sclerostin and Dickkopf-1 (DKK-1) Serum Levels in Children and Adolescents With Type 1 Diabetes Mellitus. *J Clin Endocrinol Metab* 2017; **102**: 1174-1181 [PMID: 28388723 DOI: 10.1210/jc.2016-2371]

75 **Neumann T**, Sämann A, Lodes S, Kästner B, Franke S, Kiehntopf M, Hemmelmann C, Lehmann T, Müller UA, Hein G, Wolf G. Glycaemic control is positively associated with prevalent fractures but not with bone mineral density in patients with Type 1 diabetes. *Diabet Med* 2011; **28**: 872-875 [PMID: 21395677 DOI: 10.1111/j.1464-5491.2011.03286.x]

76 **Tsentidis C**, Gourgiotis D, Kossiva L, Marmarinos A, Doulgeraki A, Karavanaki K. Sclerostin distribution in children and adolescents with type 1 diabetes mellitus and correlation with bone metabolism and bone mineral density. *Pediatr Diabetes* 2016; **17**: 289-299 [PMID: 26094958 DOI: 10.1111/pedi.12288]

77 **Wędrychowicz A**, Sztefko K, Starzyk JB. Sclerostin and its significance for children and adolescents with type 1 diabetes mellitus (T1D). *Bone* 2019; **120**: 387-392 [PMID: 30120991 DOI: 10.1016/j.bone.2018.08.007]

78 **Laurent MR**, Dubois V, Claessens F, Verschueren SM, Vanderschueren D, Gielen E, Jardí F. Muscle-bone interactions: From experimental models to the clinic? *Mol Cell Endocrinol* 2016; **432**: 14-36 [PMID: 26506009 DOI: 10.1016/j.mce.2015.10.017]

79 **Lombardi G**, Barbaro M, Locatelli M, Banfi G. Novel bone metabolism-associated hormones: the importance of the pre-analytical phase for understanding their physiological roles. *Endocrine* 2017; **56**: 460-484 [PMID: 28181144 DOI: 10.1007/s12020-017-1239-z]

80 **Boström P**, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BM. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; **481**: 463-468 [PMID: 22237023 DOI: 10.1038/nature10777]

81 **Buccoliero C**, Oranger A, Colaianni G, Pignataro P, Zerlotin R, Lovero R, Errede M, Grano M. The effect of Irisin on bone cells in vivo and in vitro. *Biochem Soc Trans* 2021; **49**: 477-484 [PMID: 33449117 DOI: 10.1042/BST20200978]

82 **Zhang J**, Valverde P, Zhu X, Murray D, Wu Y, Yu L, Jiang H, Dard MM, Huang J, Xu Z, Tu Q,

Chen J. Exercise-induced irisin in bone and systemic irisin administration reveal new regulatory mechanisms of bone metabolism. *Bone Res* 2017; **5**: 16056 [PMID: 28944087 DOI: 10.1038/boneres.2016.56]

83 **Kim H**, Wrann CD, Jedrychowski M, Vidoni S, Kitase Y, Nagano K, Zhou C, Chou J, Parkman VA, Novick SJ, Strutzenberg TS, Pascal BD, Le PT, Brooks DJ, Roche AM, Gerber KK, Mattheis L, Chen W, Tu H, Bouxsein ML, Griffin PR, Baron R, Rosen CJ, Bonewald LF, Spiegelman BM. Irisin Mediates Effects on Bone and Fat *via* αV Integrin Receptors. *Cell* 2019; **178**: 507-508 [PMID: 31299203 DOI: 10.1016/j.cell.2019.06.028]

84 **Natalicchio A**, Marrano N, Biondi G, Spagnuolo R, Labarbuta R, Porreca I, Cignarelli A, Bugliani M, Marchetti P, Perrini S, Laviola L, Giorgino F. The Myokine Irisin Is Released in Response to Saturated Fatty Acids and Promotes Pancreatic β-Cell Survival and Insulin Secretion. *Diabetes* 2017; **66**: 2849-2856 [PMID: 28724742 DOI: 10.2337/db17-0002]

85 **Colaianni G**, Faienza MF, Sanesi L, Brunetti G, Pignataro P, Lippo L, Bortolotti S, Storlino G, Piacente L, D'Amato G, Colucci S, Grano M. Irisin serum levels are positively correlated with bone mineral status in a population of healthy children. *Pediatr Res* 2019; **85**: 484-488 [PMID: 30683930 DOI: 10.1038/s41390-019-0278-y]

86 **Espes D**, Lau J, Carlsson PO. Increased levels of irisin in people with long-standing Type 1 diabetes. *Diabet Med* 2015; **32**: 1172-1176 [PMID: 25762196 DOI: 10.1111/dme.12731]

87 **Ates I**, Arikan MF, Erdogan K, Kaplan M, Yuksel M, Topcuoglu C, Yilmaz N, Guler S. Factors associated with increased irisin levels in the type 1 diabetes mellitus. *Endocr Regul* 2017; **51**: 1-7 [PMID: 28222023 DOI: 10.1515/enr-2017-0001]

88 **Faienza MF**, Brunetti G, Sanesi L, Colaianni G, Celi M, Piacente L, D'Amato G, Schipani E, Colucci S, Grano M. High irisin levels are associated with better glycemic control and bone health in children with Type 1 diabetes. *Diabetes Res Clin Pract* 2018; **141**: 10-17 [PMID: 29679630 DOI: 10.1016/j.diabres.2018.03.046]

89 **Xiong XQ**, Chen D, Sun HJ, Ding L, Wang JJ, Chen Q, Li YH, Zhou YB, Han Y, Zhang F, Gao XY, Kang YM, Zhu GQ. FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. *Biochim Biophys Acta* 2015; **1852**: 1867-1875 [PMID: 26111885 DOI: 10.1016/j.bbadis.2015.06.017]

90 **Pi M**, Quarles LD. Novel bone endocrine networks integrating mineral and energy metabolism. *Curr Osteoporos Rep* 2013; **11**: 391-399 [PMID: 24193547 DOI: 10.1007/s11914-013-0178-8]

91 **Kindblom JM**, Ohlsson C, Ljunggren O, Karlsson MK, Tivesten A, Smith U, Mellström D. Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. *J Bone Miner Res* 2009; **24**: 785-791 [PMID: 19063687 DOI: 10.1359/jbmr.081234]

92 **Zhou M**, Ma X, Li H, Pan X, Tang J, Gao Y, Hou X, Lu H, Bao Y, Jia W. Serum osteocalcin concentrations in relation to glucose and lipid metabolism in Chinese individuals. *Eur J Endocrinol* 2009; **161**: 723-729 [PMID: 19671707 DOI: 10.1530/EJE-09-0585]

93 **Pittas AG**, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. *J Clin Endocrinol Metab* 2009; **94**: 827-832 [PMID: 19088165 DOI: 10.1210/jc.2008-1422]

94 **Hygum K**, Starup-Linde J, Harsløf T, Vestergaard P, Langdahl BL. MECHANISMS IN ENDOCRINOLOGY: Diabetes mellitus, a state of low bone turnover - a systematic review and meta-analysis. *Eur J Endocrinol* 2017; **176**: R137-R157 [PMID: 28049653 DOI: 10.1530/EJE-16-0652]

95 **Madsen JOB**, Jørgensen NR, Pociot F, Johannesen J. Bone turnover markers in children and adolescents with type 1 diabetes-A systematic review. *Pediatr Diabetes* 2019; **20**: 510-522 [PMID: 30941847 DOI: 10.1111/pedi.12853]

96 **Chen SC**, Shepherd S, McMillan M, McNeilly J, Foster J, Wong SC, Robertson KJ, Ahmed SF. Skeletal Fragility and Its Clinical Determinants in Children With Type 1 Diabetes. *J Clin Endocrinol Metab* 2019; **104**: 3585-3594 [PMID: 30848792 DOI: 10.1210/jc.2019-00084]

97 **Madsen JOB**, Herskin CW, Zerahn B, Jørgensen NR, Olsen BS, Pociot F, Johannesen J. Decreased markers of bone turnover in children and adolescents with type 1 diabetes. *Pediatr Diabetes* 2020; **21**: 505-514 [PMID: 31970841 DOI: 10.1111/pedi.12987]

98 **Thrailkill KM**, Liu L, Wahl EC, Bunn RC, Perrien DS, Cockrell GE, Skinner RA, Hogue WR, Carver AA, Fowlkes JL, Aronson J, Lumpkin CK Jr. Bone formation is impaired in a model of type 1 diabetes. *Diabetes* 2005; **54**: 2875-2881 [PMID: 16186388 DOI: 10.2337/diabetes.54.10.2875]

99 **Basu R**, Peterson J, Rizza R, Khosla S. Effects of physiological variations in circulating insulin levels on bone turnover in humans. *J Clin Endocrinol Metab* 2011; **96**: 1450-1455 [PMID: 21325461 DOI: 10.1210/jc.2010-2877]

100 **Jiang N**, Xia W. Assessment of bone quality in patients with diabetes mellitus. *Osteoporos Int* 2018; **29**: 1721-1736 [PMID: 29736760 DOI: 10.1007/s00198-018-4532-7]

101 **Anagnostis P**, Paschou SA, Gkekas NN, Artzouchaltzi AM, Christou K, Stogiannou D, Vryonidou A, Potoupnis M, Goulis DG. Efficacy of anti-osteoporotic medications in patients with type 1 and 2 diabetes mellitus: a systematic review. *Endocrine* 2018; **60**: 373-383 [PMID: 29411304 DOI: 10.1007/s12020-018-1548-x]

102 **Cummings SR**, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C; FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009; **361**: 756-765 [PMID: 19671655 DOI: 10.1056/NEJMoa0809493]

103 **Kondegowda NG**, Fenutria R, Pollack IR, Orthofer M, Garcia-Ocaña A, Penninger JM, Vasavada RC. Osteoprotegerin and Denosumab Stimulate Human Beta Cell Proliferation through Inhibition of the Receptor Activator of NF-κB Ligand Pathway. *Cell Metab* 2015; **22**: 77-85 [PMID: 26094891 DOI: 10.1016/j.cmet.2015.05.021]

104 **Lasco A**, Morabito N, Basile G, Atteritano M, Gaudio A, Giorgianni GM, Morini E, Faraci B, Bellone F, Catalano A. Denosumab Inhibition of RANKL and Insulin Resistance in Postmenopausal Women with Osteoporosis. *Calcif Tissue Int* 2016; **98**: 123-128 [PMID: 26498169 DOI: 10.1007/s00223-015-0075-5]

105 **Cosman F**, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, Hofbauer LC, Lau E, Lewiecki EM, Miyauchi A, Zerbini CA, Milmont CE, Chen L, Maddox J, Meisner PD, Libanati C, Grauer A. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. *N Engl J Med* 2016; **375**: 1532-1543 [PMID: 27641143 DOI: 10.1056/NEJMoa1607948]

106 **Gil-Díaz MC**, Raynor J, O'Brien KO, Schwartz GJ, Weber DR. Systematic review: associations of calcium intake, vitamin D intake, and physical activity with skeletal outcomes in people with Type 1 diabetes mellitus. *Acta Diabetol* 2019; **56**: 1091-1102 [PMID: 30989378 DOI: 10.1007/s00592-019-01334-5]

107 **Szasz T**, Wenceslau CF, Burgess B, Nunes KP, Webb RC. Toll-Like Receptor 4 Activation Contributes to Diabetic Bladder Dysfunction in a Murine Model of Type 1 Diabetes. *Diabetes* 2016; **65**: 3754-3764 [PMID: 27650857 DOI: 10.2337/db16-0480]

108 **Vijayan V**, Khandelwal M, Manglani K, Gupta S, Surolia A. Methionine down-regulates TLR4/MyD88/NF-κB signalling in osteoclast precursors to reduce bone loss during osteoporosis. *Br J Pharmacol* 2014; **171**: 107-121 [PMID: 24111943 DOI: 10.1111/bph.12434]

109 **Cao Y**, Han X, Wang Z, Liu Y, Wang Y, Zhang R, Ye J, Zou L, Dai W. TLR4 knockout ameliorates streptozotocin-induced osteoporosis in a mouse model of diabetes. *Biochem Biophys Res Commun* 2021; **546**: 185-191 [PMID: 33601314 DOI: 10.1016/j.bbrc.2021.01.102]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1010-1025

DOI: 10.4239/wjd.v12.i7.1010

ISSN 1948-9358 (online)

REVIEW

Current cancer therapies and their influence on glucose control

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Author contributions: Yim C, Mansell K, Hussein N and Arnason T contributed to the research and writing of distinct sections of the paper; Arnason T and Mansell K edited the manuscript.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

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Abstract

This review focuses on the development of hyperglycemia arising from widely used cancer therapies spanning four drug classes. These groups of medications were selected due to their significant association with new onset hyperglycemia, or of potentially severe clinical consequences when present. These classes include glucocorticoids that are frequently used in addition to chemotherapy treatments, and the antimetabolite class of 5-fluorouracil-related drugs. Both of these classes have been in use in cancer therapy since the 1950s. Also considered are the phosphatidyl inositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR)-inhibitors that provide cancer response advantages by disrupting cell growth, proliferation and survival signaling pathways, and have been in clinical use as early as 2007. The final class to be reviewed are the monoclonal antibodies selected to function as immune checkpoint inhibitors (ICIs). These were first used in 2011 for advanced melanoma and are rapidly becoming widely utilized in many solid tumors. For each drug class, the literature has been reviewed to answer relevant questions about these medications related specifically to the characteristics of the hyperglycemia that develops with use. The incidence of new glucose elevations in euglycemic individuals, as well as glycemic changes in those with established diabetes has been considered, as has the expected onset of hyperglycemia from their first use. This comparison emphasizes that some classes exhibit very immediate impacts on glucose levels, whereas other classes can have lengthy delays of up to 1 year. A comparison of the spectrum of severity of hyperglycemic consequences stresses that the appearance of diabetic ketoacidosis is rare for all classes except for the ICIs. There are distinct differences in the

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 7, 2021
Peer-review started: February 7, 2021
First decision: March 16, 2021
Revised: April 11, 2021
Accepted: June 25, 2021
Article in press: June 25, 2021
Published online: July 15, 2021

P-Reviewer: Park JH, Vela D
S-Editor: Liu M
L-Editor: A
P-Editor: Wang LL

reversibility of glucose elevations after treatment is stopped, as the mTOR inhibitors and ICI classes have persistent hyperglycemia long term. These four highlighted drug categories differ in their underlying mechanisms driving hyperglycemia, with clinical presentations ranging from potent yet transient insulin resistant states [type 2 diabetes mellitus (T2DM) -like] to rare permanent insulin-deficient causes of hyperglycemia. Knowledge of the relative incidence of new onset hyperglycemia and the underlying causes are critical to appreciate how and when to best screen and treat patients taking any of these cancer drug therapies.

Key Words: Cancer therapy; Hyperglycemia; adverse drug effects; Immune checkpoint inhibitors; mTOR inhibitors; 5-fluorouracil analogs; Glucocorticoids; Diabetes mellitus

Core Tip: Immune checkpoint inhibitors (ICI) rarely cause hyperglycemia, but glucose monitoring from their initiation is critical as rapid diabetic ketoacidosis can develop from underlying immune-mediated pancreatic beta-cell destruction. Therapy with mammalian target of rapamycin (mTOR) inhibitors, 5-fluorouracil (5-FU)-analogs and glucocorticoids have higher rates of hyperglycemia early in therapy that is not generally severe, but needs to be recognized and treated to optimize patient outcomes. The hyperglycemia occurring from the 5-FU and ICI classes is not reversible. The diabetes from ICIs arises from an absolute insulin deficiency *vs* the partial deficiency from the 5-FU class. Glucocorticoids and mTOR inhibitors predominantly cause insulin resistance.

Citation: Yim C, Mansell K, Hussein N, Arnason T. Current cancer therapies and their influence on glucose control. *World J Diabetes* 2021; 12(7): 1010-1025
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1010.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1010

INTRODUCTION

Cancer therapies have had profound impacts on increased life expectancy over the past few decades, however, it is widely known to have a multitude of unintended effects. Quality of life concerns such as hair loss, intractable nausea or visible surgical scars are widespread in individuals initiating their treatment cycles. Physicians initiating chemotherapy are also concerned about treatment side effects and routinely monitor for signs or symptoms of serious complications that may require urgent hospitalization, a change in treatment management or a pause in therapy to avoid a life-threatening event. Hyperglycemia is a common and potentially significant adverse effect arising from the use of several widely applied cancer therapeutic classes including immune checkpoint inhibitors (ICIs), phosphatidyl inositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) inhibitors, 5-fluorouracil (5-FU) analogs, and glucocorticoids[1-4]. The latest understanding of the characteristics of the hyperglycemia that is associated with the use of these drug classes is presented in order to raise awareness of the adverse effects these agents have on glucose control to enable its early recognition, trigger regular monitoring plus timely intervention, and to ultimately improve patient outcomes. Emphasized below is the current knowledge pertaining to these drug classes regarding the incidence, onset, reversibility and severity of hyperglycemia associated with their use in cancer therapy.

The significance of hyperglycemia on cancer therapy outcomes

Untreated hyperglycemia has been associated with a multitude of negative outcomes for cancer patients including longer hospital stays[5], worsened prognosis and decreased survival[6,7]. Glucose is a key substrate metabolized by cells to produce ATP and is a preferred energy supply; cancer cells are known to increase their glucose uptake, with the subsequent increase in energy reserves able to support further cellular proliferation[8]. Hyperglycemia has also been associated with a reduction in

cancer therapy effectiveness[9], an increased rate of infections and sepsis in those who may already have immunosuppression from their cancer treatments[10], and an increased length in hospital[11,12]. Hyperglycemia fosters a proinflammatory environment that enhances the production of cancer stimulating signals that promote cell proliferation, increase resistance to cell death and may also induce drug resistance to chemotherapy[11-16]. Clinically, hyperglycemia has been found to be an independent risk factor for earlier cancer recurrences, and higher mortality rates[17].

Glucose levels and clinical presentation define the severity of hyperglycemia

The research referenced below has graded both the severity of hyperglycemia and the degree of clinical symptoms as a means of comparing patient adverse events (AE) with drug use. Four grades of severity are defined that consider glucose levels, but also includes the severity of the clinical consequence such a diabetic ketoacidosis (DKA) or permanent diabetes. Grade 1 AE (G1) relates to asymptomatic or mild symptoms, no ketosis or evidence of type 1 diabetes (T1DM), fasting glucose (FG) above normal. Grade 2 AE (G2) involves moderate symptoms, FG > 8.9-14 mmol/L, or the presence of ketosis or T1DM at any glucose level. Grade 3-4 AE are severe symptoms, that are medically significant or life-threatening, differentiated from G2 and each other by the degree of glucose elevations with Grade 3 AE (G3) encompassing glucose levels between 13.9-27.8 mmol/L, and Grade 4 AE (G4) including glucose levels > 27.8 mmol/L[18].

IMMUNE CHECKPOINT INHIBITORS

Immune Checkpoint inhibitors target one of three T-cell ligands to promote antitumor activity

A relatively new class of chemotherapy agents that are recognized for their potential side effects on glucose control are the immunomodulators that target and inhibit immune checkpoints, resulting in an increase in T-cell mediated immune responses that benefit patient treatment responses[19]. These ICIs are monoclonal antibodies that bind and block (inhibit) immune cell-cell interactions that would normally suppress the immune response. The result is that there is an effective and durable increase in antitumour activity[20]. This class is very successful in the treatment of advanced melanoma including those with *BRAF* mutations[21], and have since been used successfully for treatment of additional advanced stage cancers including hepatocellular carcinoma[22,23], non-small-cell lung cancer[24], renal cell carcinoma[25] and metastatic clear cell renal cancer[26]. The ICIs in current use specifically block three T-cell checkpoints; the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) receptor, the programmed cell death-1 (PD-1) receptor, and the third and most recent class of antibodies targeting the programmed cell death-Ligand 1 (PD-L1)[19,20].

ICIs trigger immune-related endocrinopathies, and the incidence of diabetes with ICI differs with the checkpoint being targeted

There is an association between the use of these ICIs and the frequent appearance of immune-related AE, including a wide-spectrum of endocrine dysfunctions. The onset of hyperglycemia in individuals taking ICIs is infrequent, and the incidence differs depending on the receptor being targeted, as well as whether receptor targeting combinations are used[27]. Not all ICIs appear to have the same potential. The highest probability appears to reside in those targeting either the PD-1 receptor (nivolumab, pembrolizumab) or PD-L1 (atezolizumab, durvalumab, avelumab), whereas the CTLA-4 targeting agent (ipililmumab) does not seem to have a significant risk when used alone, as only a handful of case reports were noted[28]. A recent 2020 meta-analysis estimated the incidence of serious (G3 and G4) and all-grade hyperglycemia (G1-G4) in every reported case of ICI-associated diabetes, noting that the PD-1/PD-L1 targeted therapies were associated with hyperglycemia in 0.2%-4.9%, with a 0.49% incidence of serious hyperglycemia in patients using these drugs[1]. A 2018 study reported an overall incidence of 0.9%[28]. The combination of PD-1/PD-L1 and CTLA-4 immune-targeted therapy showed the highest overall rates of diabetes, spanning 2.0%-3.4% in different cohorts studies, and a notably higher rate of serious hyperglycemia events at almost 2%[28]. This same study confirmed that the CTLA-4 inhibitors do not seem to have a risk of hyperglycemia when used without PD-1 therapies[28].

ICI therapy stimulates immune-mediated antitumor activities, but also stimulates autoimmune disorders

The inhibitory monoclonal antibodies used to interrupt immune response checkpoints results in a reinvigoration of the immune response. The ICI antibodies bind and block the specific inhibitory ligand on the T-cell surface, interrupting those activity-dampening signaling pathways[29,30]. The result is T-cell activation and stimulation of their immune surveillance and antitumor activity, to the benefit of the patient[31]. However, immune checkpoints are also central to maintaining immunological self-tolerance and preventing autoimmune disorders[32,33]. Immune-mediated self-damage causing endocrine dysfunctions are one of the most common side effects of the ICI class, including loss of thyroid, adrenal and pituitary activity, plus rare cases of pancreatic insulin deficiency[34]. Autoimmune recognition and destruction of pancreatic beta (β)-cells is the well-established mechanism resulting in classic T1DM [35,36], and the ICI drugs likely trigger this same destructive loss of function in cancer patients who developed hyperglycemia[37]. Human pancreatic islets lack CTLA-4 receptors, but do present PD-L1 to protect them against immune cells[38]. The ICI monoclonal antibodies that bind PD-1/PD-L1 should be capable of inhibiting this pathway in pancreatic β-cells, leaving them susceptible to (auto)immune destruction and diabetes, providing a rationale why PD-1/PD-L1 but not CTLA-4 inhibitors are associated with new onset diabetes.

The hyperglycemia associated with ICI use is due to autoimmune destruction of the pancreatic ß-cells and loss of endogenous insulin release

With ICI use, the new onset of hyperglycemia found in those without diabetes, and the worsening glucose control in those with known diabetes, does appear to be directly due to immune-mediated pancreatic damage. Pancreatitis was found in 42% of individuals developing diabetes, and auto-antibodies classically associated with T1DM can be found elevated in these individuals, with 47% having glutamate decarboxylase autoantibodies[28]. The appearance of new hyperglycemia in those exposed to ICI therapy is not caused by an associated insulin resistance, as three large case series evaluating patients that developed diabetes after ICI exposure found low C-peptide (62%-93%), positive ketosis (59%-77%) and detectable autoantibodies (39%-56%)[28,37,39], with the antibodies in at least some cases not present prior to ICI treatment[28].

Loss of glucose regulation in type 2 patients taking ICIs may indicate a transformation into an insulin-deficient state

It is less well known how ICI use has impacted glucose levels in those with underlying T2DM as the stress of illness, pain, or other medical therapies may also contribute to loss of tight glucose control. It is well documented, however, that when blood glucose levels become acutely and significantly more difficult to control in known T2DM, that it is important to consider that the ICI therapy may have caused pancreatic β-cell dysfunction and insulin deficient diabetes[40].

The onset of Insulin-deficient diabetes after ICI therapy is unpredictable and is permanent

Of the cases of insulin-deficient diabetes (IDD) reported with ICI therapy, the onset is unpredictable and can appear as early as a few weeks after starting treatment, up to greater than one-year following therapy; over half occurred within 4 mo of treatment initiation, typically in their fourth cycle of therapy[28]. The presence of hyperglycemia with ICI therapy does not require cessation of the ICIs or provision of high dose steroid pulse therapy, as this does not appear to restore pancreatic function[41,42]. In fact, reversal of IDD after ICI use has rarely been reported. A single case of ICI-induced diabetes successfully used infliximab, an immunosuppressant, to reverse the hyperglycemia[43], yet in general, once present the hyperglycemia is persistent and does not appear to be mitigated by decreasing or stopping the ICI treatment[34,44].

ICI associated hyperglycemia has a high risk of serious and severe consequences, notably DKA and permanent diabetes

ICI therapy can lead to severe complications of hyperglycemia that can occur very rapidly. The severity is due to the damage to the pancreatic β-cells, leading to irreversible insulin deficiency. Because of this T1DM-like defect, there is a distinct risk of DKA, and this can be an acute and potentially life-threatening presentation. The association between ICI-dependent onset of hyperglycemia and ketosis/DKA was

remarkably high, at 77.8 % in newly diagnosed cases of diabetes[37]. There are many case reports of rapid DKA as the first presentation of hyperglycemia with ICI use, raising the possibility that this overlaps with Fulminant T1DM, a clinical presentation that is characterized by rapid development of markedly elevated glucose, near-normal glycated hemoglobin A1c (A1C), ketoacidosis, negative autoantibodies, severe insulin deficiency and elevated levels of pancreatic enzymes[45]. A careful review, however, revealed that there does appear to be distinct differences, including the presence of autoantibodies in ICI IDD, that are typically not found in Fulminant T1DM[37]. Due to the risk of DKA with this drug class, the practice guidelines developed to monitor for adverse effects of ICIs commonly recommend routine monitoring of glucose levels both at baseline, with each treatment cycle throughout induction and then every 3-6 wk thereafter for up to one year[18]. For safety, the use of insulin for diabetes developing from ICI therapy is recommended unless insulin deficiency can be ruled out[18].

PI3K/AKT/MTOR PATHWAY INHIBITORS

Inhibition of the PI3K/AKT/mTOR pathway interrupts multiple cancer promoting cell signals

The PI3K-AKT-mTOR signaling pathway plays a vital role in responding to nutrient abundance[46], making it an attractive target for blockade[47]. The proteins being inhibited are kinases that target downstream proteins for phosphorylation to change cellular responses including promoting normal cell growth and proliferation when nutrients are abundant[48]. They ultimately work within the same pathway as growth factors and insulin signaling, and can therefore also influence glucose and lipid metabolism[49].

mTOR, PI3K inhibitors and their derivatives are effective in many cancer types

mTOR inhibitors are derived from the original drug of this family, rapamycin, that was initially isolated as an antifungal agent[50], but was later determined to inhibit a kinase important in cancer growth[51]. This target was subsequently named "mechanistic target of rapamycin" or mTOR. mTOR inhibitors and their related analogs are used in many advanced stage solid tumors including renal cell, neuroendocrine tumors of the pancreas, and breast cancer[52]. There are presently three mTOR inhibitors approved by the United States Food and Drug Administration (FDA) that are derivatives of rapamycin; sirolimus, temsirolimus, and everolimus. Closely related medications are the PI3K inhibitors, of which there are four currently approved by the FDA; copanlisib, idelalisib, duvelisib, and alpelisib. These latter agents are approved for use in the treatment of breast cancer and hematological malignancies. AKT inhibitors and combination PI3K/mTOR inhibitors are still under development and some have entered Phase II clinical trials[53].

The hyperglycemia arising from the inhibition of mTOR is primarily due to insulin resistance

The usual activity of mTOR not only influences cell growth and development, but also affects glucose regulation[54]. mTOR inhibitors primarily promote hyperglycemia through increased insulin resistance *via* mTOR complex 1 (mTORC1) inhibition, as they impair the efficiency of the insulin signaling pathway at multiple points in its phosphorylation cascade[55,56]. In a diabetic rodent model, exposure to rapamycin resulted in a reduction in insulin signaling *via* proteins IRS1/2, a reduction in phosphorylation by AKT, and inhibition of PI3K activity[55]. Moreover, rapamycin increased the activation of Jun N-terminal kinase pathway, which is a pathway implicated in insulin resistance[55]. Together, the effect observed with these chemotherapy drugs is consistent with a predominant T2DM-like insulin resistant state, due to impaired insulin signaling[55]. Lastly, a component of insulin deficiency is also thought to play a role in the development of hyperglycemia as mTORC1 is a known positive regulator of pancreatic β-cell function, and molecular studies using pancreatic β-cells exposed to rapamycin detected a 33% reduction in glucose-induced insulin secretion[55].

There are two mTOR complexes that differ in their influence on glucose levels and sensitivity to inhibition

The mTOR complex is a serine/threonine protein kinase that exists in two different multi-protein complexes. The mTORC1 is sensitive to rapamycin, whereas complex 2 (mTORC2) is less responsive to rapamycin, although chronic exposure to rapamycin does ultimately result in reduced mTORC2 signaling[56]. The mTORC2 pathway is much less well characterized than the mTORC1 pathway. It was initially thought that the mTORC2 pathway was resistant to rapamycin treatment, but it was later discovered that long term exposure reduces mTORC2 signaling in some cell types by suppressing the assembly of the mTORC2 complex[57]. mTORC2 activates AKT, and the mTORC2-AKT pathway has been shown to promote pancreatic beta cell proliferation and survival, and to inhibit gluconeogenesis by blocking Fox01 activity[57]. Normal mTORC2-AKT activity also induces glucose uptake in insulin-sensitive tissues and blocks protein catabolism. The loss of mTORC2 activity through inhibition, therefore, increases insulin resistance as well as promoting protein catabolism and reducing muscle mass. Inhibition of mTORC2 also leads to the loss of the mTORC2-AKT-dependent inhibition of gluconeogenesis as well as decreased insulin production, contributing further to hyperglycemia[56]. The effect of mTORC1 and mTORC2 inhibition on glycemia is complex, and related to the degree and chronicity of inhibition, but ultimately treatment with all mTOR inhibitors leads to hyperglycemia [56]. The three mTOR inhibitors approved by the FDA are derivatives of rapamycin; sirolimus, temsirolimus, and everolimus, and are primarily mTORC1 inhibitors, although dual mTORC1/C2 inhibitors are in development[57].

The PI3K/AKT/mTOR pathway inhibitors are potent drivers of hyperglycemia

The incidence of hyperglycemia associated with the use of PI3K/AKT/mTOR inhibitors is significant and ranges between 12%-50%[2]. A 2015 meta-analysis considered twenty-four trials of mTOR inhibitor use in solid organ cancer treatment and noted a 5.25-fold increased risk of significant hyperglycemia (blood sugars > 14 mmol/L)[58]. Pre-existing diabetes was an independent risk factor for glucose levels > 14 mmol/L[59]. It is worth noting that the PI3K inhibitors can also induce hyperglycemia[60], and AKT inhibitors have revealed significant hyperglycemia in preclinical studies[61].

Most cases of hyperglycemia occur during initial exposure, are mild and transient

A retrospective study of 341 patients treated with PI3K and mTOR inhibitors revealed that the mean FG increased from 5.3 mmol/L at baseline to 7.1 mmol/L during the first chemotherapy cycle, but returned to 5.4 mmol/L prior to the next cycle[59]. This supports the conclusion that the rise in blood glucose is transient. The majority of these patients experienced their highest glucose levels early on in therapy, during the first (87.9%) or second (14.4%) cycle of mTOR inhibitor treatment, and most cases of hyperglycemia in this study were mild (G1)[59]. However, more significant glucose elevations can occur, as 6.7% of patients receiving this therapy had glucose elevations > 14 mmol/L compared to controls not taking mTOR inhibitors[62]. Additionally, it was observed that the median time of elevated glucose levels (> 8.3 mmol/L) was 56 d in patients showing clinical benefit, and 113 d for those patients who progressed[62]. It remains to be determined if the timing of new hyperglycemia development after therapy initiation is predictive of treatment responses.

mTOR-induced hyperglycemia is typically managed with oral therapies

Insulin deficiency or DKA are not significant risks with using this class of drugs, as only a very small percentage of patients require insulin[59]. and to our knowledge there have been no cases of hyperglycemic emergency or DKA in any clinical trials to date. A single case report was found that describes DKA and pancreatitis in a patient treated with everolimus for breast cancer, supporting that this is a very rare association with mTOR inhibitor drugs[63]. When uncontrolled hyperglycemia develops (defined as glucose > 14 mmol/L, A1C ≥ 9%), expert committee guidelines recommend stopping the chemotherapy medication and reintroducing it a lower dose in the rare cases of uncontrolled hyperglycemia despite optimal diabetes management [2]. Both American and French guidelines for PI3K/AKT/mTOR use are available to direct surveillance and treatment best practices[2,64], and an A1C target of ≤ 8% is suggested for pre-existing patients with diabetes prior to mTOR inhibition[2].

5-FU AND DERIVATIVES

5-FU is an antimetabolite agent that has been used in the initial treatment of breast, gastric, colon and pancreatic cancers and has been in active use for over sixty years[65,66]. It is a pyrimidine analogue that is structurally related to thymine, uracil and cytosine bases in DNA, RNA or both, respectively[67]. 5-FU acts as an antimetabolite to inhibit cell growth through its interference with DNA and RNA function upon its incorporation into newly synthesized DNA or RNA.

Derivatives of 5-FU have been created to increase their stability and to overcome their drug toxicities

Over the years, additional 5-FU oral prodrugs have been developed that reduce their toxicity and improve tumour selectivity, as well as increase their stability[68-70]. In the last 20 years, capecitabine has been developed and used predominantly for metastatic breast and gastrointestinal cancers[66,70]. It is activated into 5-FU through three sequential enzymes, with the final enzyme being found in high concentrations in tumour tissues[66]. As such, capecitabine activation is very targeted and is generally better tolerated[66,70]. There have been numerous reports of glucose disorders with 5-FU and its derivatives including case reports of hyperglycemia following the administration of the newest 5-FU prodrug, capecitabine[71].

5-FU prodrugs can contribute to new onset diabetes, and the majority have persistent hyperglycemia after therapy is stopped

There is a paucity of data on 5-FU therapies and their specific effects on glucose control. The majority of information comes from a 2013 study involving 362 patients with normal fasting plasma glucose prior to 5-FU-based therapies in which overt diabetes developed in 11.6% of individuals and impaired fasting glucose (IFG) in another 11.3%[3]. Of the 42 patients that developed diabetes, 32 occurred during therapy, with the remaining 10 being detected during follow-up after treatment was completed. Only 16% (7/42) of these patients had glucose levels spontaneously return to normal[3], indicating that the hyperglycemia related to 5-FU therapy is persistent in most cases. Those remaining were managed with a variety of interventions including diet (30%), insulin (10.8%) or oral medications[3]. Given that these patients did not have pre-existing risk factors for diabetes, it was thought that the development of diabetes was secondary to 5-FU chemotherapy[3].

Hyperglycemia typically develops early during 5-FU analog therapy, and is generally mild

The timing of new-onset hyperglycemia with 5-FU treatments varies, but most (77%) patients developed diabetes during their early chemotherapy cycles (median third cycle), and the remaining individuals present up to 1 year after completion of treatment[3]. It is unclear how 5-FU chemotherapy affects glucose control in those with established diabetes. In this study, the timing of the onset of IFG after 5-FU treatment was not reported[3]. While 5-FU-associated hyperglycemia was typically mild during active treatment (95% had glucose < 14 mmol/L), after therapy was complete it was noted that seven out of 42 patients developed significant hyperglycemia (>14 mmol/L), and one patient in the study died of ketoacidosis[3]. Aside from this study, there are two additional case reports of DKA associated with 5-FU based treatments [72,73].

5-FU therapies decrease pancreatic β-cell insulin storage and release

The underlying mechanism causing the hyperglycemia upon 5-FU exposure appears to be due to a decrease in insulin being released from the pancreatic β-cells[3,74]. Those patients who developed diabetes had a progressive decrease and delay in C-peptide secretion, seemingly due to a pancreatic deficiency in endogenous insulin processing and production[3]. A case control study also demonstrated that insulin levels failed to increase appropriately with the development of hyperglycemia[74]. Preclinical animal studies also suggest that hyperglycemia may result from impaired insulin production as there was a relative insulin deficiency in rats following 5-FU administration, as well as a decrease in the abundance of secretory granules in pancreatic islet cells[75]. Cellular studies designed to reveal how these drugs cause hyperglycemia have shown that 5-FU related therapy stimulates immune mediators in pancreatic β-cells, resulting in their destruction *via* cell-mediated T-cell infiltration[76]. Consistent with this, capecitabine has been linked to acute pancreatitis[77,78].

The rare cases of DKA reported with 5-FU therapies suggests that there is sufficient endogenous insulin production to offset severe hyperglycemia consequences in the majority of cases. Nonetheless, there is a real risk of significant glucose elevations, as 16.7% (7 of 42) of newly diabetic individuals had glucose levels > 14 mmol/L despite therapy[3]. Management of hyperglycemia following capecitabine therapy included successful treatment with dietary control and lifestyle changes[79,80], although some individuals did require insulin[3].

GLUCOCORTICOIDS

Glucocorticoids are a class of medications that have been used to treat a plethora of medical conditions since the 1950's. Glucocorticoids are prescribed widely for a variety of medical conditions, with estimates of use approaching 1% of the general population [81]. Although their efficacy and adverse effect profile have been described extensively in the literature, their effect on the human body varies due to the heterogeneous nature of the underlying disease states they are treating and the individuals who are using them; hence there is variability in their use and dosage recommendations[82].

Steroids are useful as adjunct therapy to offset adverse side effect of cancer treatments

Glucocorticoids are often included as a part of cancer therapy to mitigate the adverse effects of the chemotherapies being used at the same time. They can be very useful in controlling nausea and improving appetite, and are frequently given as an antiemetic before and after chemotherapy[83]. The dosing and duration often depends on the emetogenic potential of the chemotherapy. Glucocorticoids are also given to prevent some of the other adverse effects of chemotherapy like generalized rash or thrombophlebitis when drugs are given through peripheral vein, or to offset hypersensitivity reactions[11,84,85]. Glucocorticoids may also be included as an inherent part of the cancer therapy, such as their use within the CHOP protocol in lymphoma. There are several other regimens used in multiple myeloma and prostate cancer that include glucocorticoids as a part of the treatment, and the dosing and formulation varies.

Glucocorticoid-induced hyperglycemia is a very common adverse effect of steroid use

Along with their known benefits, there are many recognized adverse effects of glucocorticoids, both acute and chronic. Supraphysiologic glucocorticoid use is known to raise glucose levels, particularly at the high doses that are required for therapeutic advantages. Glucocorticoid-induced hyperglycemia (GIH) is a well-known complication of their use in individuals with known diabetes (T1DM and T2DM) as well as those who were previously euglycemic[4,86]. Hyperglycemia is commonly reported in patients undergoing cancer therapy that includes glucocorticoids, however its true incidence is hard to define due to the variability in chemotherapy combinations, durations, and cycles. One study of hospitalized patients taking high dose glucocorticoids reported hyperglycemia in 52% of patients[87], with another two studies reporting 34%[88] and 37%[89] in patients during induction therapy for acute lymphocytic leukemia[88,89]. A more recent study found that 94% of women with gynecological cancer whose chemotherapy regimen included high dose dexamethasone experienced hyperglycemia[85]. These patients were undergoing continuous glucose monitoring (CGM) which the authors felt led to the remarkably high incidence rate, and postulated that glucose elevations may be significantly under-recognized in many previous clinical trials that did not utilize CGM[85].

GIH occurs acutely and is generally mild

GIH is a phenomenon that typically occurs acutely with initiation[85,87,90,91], and hyperglycemia was found to be significant by day 2 in those being treated systemically for hematologic malignancies[11]. The degree of glucose elevations range widely and are most frequently modest (< 14 mmol/L)[92], nonetheless severe hyperglycemia (> 28 mmol/L) and DKA have also been reported[11,93], with rare reports of hyperglycemic hyperosmolar syndrome as well[94]. Other AE associated with GIH range from mild to serious, such as increased infections and lengthened of hospital stays[11,16,86,95-98]. The acute hyperglycemia associated with glucocorticoids will typically resolve upon discontinuation[4,99,100].

The formulation and duration of steroid use influences the incidence of hyperglycemia

The most commonly used glucocorticoids in chemotherapeutic regimens are prednisone (oral) and dexamethasone (oral or intravenous). The dose and duration at which they are used varies with the chemotherapy and clinical situation, making general conclusions difficult[16]. To give an example of the variance that confounds these clinical assessments, a study by Ochola *et al*[95] considered patient outcomes with prednisone use; there was a range in total daily doses of 40-150mg; once to four times daily; and between 5-14 d duration.

It is commonly considered that higher glucocorticoid doses and longer durations of use confers a greater risk of developing GIH[11,16,101,102], yet there have been exceptions to this association[82,94,98]. Most hospitalized patients developed hyperglycemia after taking ≥ 40 mg/d of prednisone for two days[103]. There is some evidence that splitting the dose of prednisone, rather than administering it all at once in the morning, may help reduce GIH[104]. The type of glucocorticoid used may also correlate with the risk of hyperglycemia[92]. Healy *et al*[11] found that hyperglycemia was associated with higher doses and the longer-acting steroids in those without diabetes, yet it was not in those with previous diabetes.

Due to the differences in the pharmacokinetic profiles of shorter acting glucocorticoids (such as prednisone, prednisolone, and hydrocortisone) *vs* longer-acting glucocorticoids such as dexamethasone, one could anticipate a delayed effect with the latter[90]. Prednisone levels peak 4-8 h after ingestion and its duration of action is between 12 h to 16 h; these pharmacokinetics correlated with increases in postprandial glucose in the afternoon and evening when administered in the morning[11,87,92]. During induction therapy for acute lymphoblastic leukemia the use of long-lasting dexamethasone was linked with a significant increase in risk of GIH when compared to those prescribed the intermediate-acting prednisone[93]. In contrast, a comparison between dexamethasone 8-12mg IV and prednisone 40mg orally found extensive hyperglycemia in the majority of all patients, without differences between the two therapies[94].

Steroids induce a potent insulin resistance resulting in hyperglycemia

The effects of glucocorticoids on glucose levels are complex[4]; although GIH occurs most commonly in patients with pre-existing diabetes, it also presents in those without any prior history of hyperglycemia[82,105]. Glucocorticoids can cause an increase in both fasting and postprandial glucose levels, but it is generally recognized that the largest impact is on postprandial levels[16,87,92,95,96,105,106]. High dose glucocorticoid use impairs insulin signaling, leading to key increases in insulin resistance at the liver (promoting hepatic gluconeogenesis) and skeletal muscle (impairing glucose uptake)[4,92,106]. Glucocorticoids can also diminish normal insulin secretion by pancreatic β-cells[4,99]. Some of the predictors of risk for increased blood glucose with glucocorticoid use in the context of cancer therapy include older age and higher BMI [88,98,102] and while an elevated A1C was found to be a predictor, a discrete HbA1c cut-off was not determined[85].

SUMMARY AND DISCUSSION

For all of these classes of drugs, it would be prudent to initiate glucose monitoring upon the initiation of chemotherapy and to continue to do so throughout treatment. As summarized in Table 1, glucocorticoids and AKT/mTOR inhibitors can be expected to cause the majority of patients (up to 94%[85] and 50%[2] respectively) to develop hyperglycemia very early after drug initiation. In contrast, the diabetes that develops upon the initiation of ICIs and 5-FU therapies will affect fewer individuals (up to 5%[1] and 11%[3], respectively) and could be anticipated to present at slightly later timelines on average, with the 5-FU analogs typically in their third chemotherapy cycle[3] and ICIs in their fourth chemotherapy cycle (about 4 mo[28]). Despite searching the literature, it was not found that there are dosing 'cut-offs' for any drug class below which the risk of hyperglycemia is nil, nor are there specified doses above which there are significantly increased rates of hyperglycemia.

The insulin resistance arising from either glucocorticoids or AKT/mTOR inhibitors nearly always resolved once the treatments have stopped[4,59,99,100] and there have not been any reports of delayed reappearance, implying that ongoing daily glucose monitoring will not be necessary upon completion (Table 2). When mild hyperglycemia is present, standard management approaches used for T2DM have been

Table 1 Summary of reported characteristics of hyperglycemia incidence, onset and severity with the use of current chemotherapy agents

Characteristics by drug class	Glucocorticoids	5-FU and analogs	PI3K/mTor inhibitors	Immune checkpoint inhibitors
Incidence of new or worsening hyperglycemia	Significant, 34%-94%	Common, 11.6% DM, 11.3% IFG	Significant, 12%-50%	Rare, 0.2%-4.9%
Onset of hyperglycemia after first use	Acutely	Majority by 3 mo; 3/4 early (3rd cycle); 1/4 up to 1 yr later	Majority after first use	Majority by 4 mo, can be after first use, can be up to 1 yr later
Severity of hyperglycemic events	Usually mild, Severe possible, Multiple reports of DKA and some HHS	Mild, Case reports of DKA	Mild, No DKA	Moderate to severe, 77.8% DKA

5-FU: 5-fluorouracil; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; PI3K: Phosphatidyl inositol-3-kinase; mTOR: Mammalian target of rapamycin; DKA: Diabetic ketoacidosis; HHS: Hyperglycemic hyperosmolar syndrome; IFG: Impaired fasting glucose.

Table 2 Hyperglycemia can be a class or drug-specific effect and may not be reversible with discontinuation

Characteristics by drug class	Glucocorticoids	5-FU and analogs	PI3K/mTOR inhibitors	Immune checkpoint inhibitors
Class effect on hyperglycemia	Yes	Yes	Yes	Negligible risk with the CTLA-4 inhibitor, ipililmumab Does occur with all PD-1 and PD-L1 inhibitors, most significantly when combined
Reversibility of hyperglycemia	Yes	No	Yes	No

5-FU: 5-fluorouracil; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; PI3K: Phosphatidyl inositol-3-kinase; mTOR: Mammalian target of rapamycin; PD-1: Programmed cell death-1; PD-L1: Programmed cell death-Ligand 1.

effective including diet adjustments, oral metformin or sulfonylureas[2,64] (Table 3). While there is little information specifically related to all of these drug classes, it would be anticipated that DPP-4 inhibitors, SGLT-2 inhibitors, or GLP-1 analog medications would also be effective at normalizing blood glucose levels. Increases to therapy intensiveness to manage more severe glucose elevations would follow usual best practices for T2DM management, and patients may ultimately require insulin for optimal control in the short term.

The ICIs and 5-FU analog classes cause hyperglycemia due to varying degrees of insulin deficiency at the level of the pancreatic β-cell, and once present the diabetes is generally permanent[3,34,44] (Table 2). As discussed above, it is very important to continue glucose monitoring even after therapy has been completed with these two classes, as diabetes can develop for up to at least one year. To date, there is little direction surrounding the specific monitoring and management of hyperglycemia in patients treated with 5-FU, in contrast to multiple current guidelines available for the numerous autoimmune adverse effects of ICI, including IDD[18] (Table 3).

The diabetes developing as a result of 5-FU analog therapies has rarely led to DKA, suggesting that the insulin deficiency is not absolute in the great majority of cases (Table 1). This raises the possibility that sulfonylureas may have a beneficial role in mild glucose elevations due to their ability to enhance pancreatic β-cell insulin secretion; this increased release of insulin may compensate for the underlying low insulin levels and thereby normalize blood glucose levels. This has neither been specifically investigated nor reported, but their use could be rationalized based on the underlying defect driving hyperglycemia with 5-FU therapy.

Independent of ICI therapy, the majority of T1DM occurs in children or young adults, and there is a global all-age incidence of 15 per 100000 persons[107]. The 0.2%-4.9% incidence of insulin-deficient hyperglycemia in adults after ICI therapy (median age > 60 years years[1]) is higher than global rates and presents in older than expected age groups, suggesting that its development may be more complex than merely unmasking those at inherent risk for developing T1DM. Without doubt there are complexities not yet appreciated, yet it is not known how to identify those at highest risk for the development of T1DM with ICI use. As these therapies become more

Table 3 The underlying mechanisms and treatment considerations of hyperglycemia differ between chemotherapy classes

	Etiology of hyperglycemia	Treatment considerations
Glucocorticoids	Major: Insulin resistance	Oral hypoglycemics possible for mild
	Minor: Decreased insulin release	Consider selecting insulins with duration of action to match that of the steroid being given
5-FU and analogs	Major: Decreased insulin release and production	Diet or oral hypoglycemics for mild
		Insulin for severe
PI3K/mTOR inhibitors	Major: Insulin resistance	Diet or metformin for mild
Immune checkpoint inhibitors	Major: Profound insulin deficiency	Immediate initiation of insulin in new onset hyperglycemia
		Switch to insulin in pre-existing T2DM

5-FU: 5-fluorouracil; PI3K: Phosphatidyl inositol-3-kinase; mTOR: Mammalian target of rapamycin; T2DM: Type 2 diabetes mellitus.

widespread and cases rise, it may become more clear. At this time, it has been considered that the HLA-DR4 genotype and presence of other autoimmune diseases may correlate with increased risk[28].

For the ICI class, insulin therapy is essential in new onset diabetes given the extreme risk of IDD causing severe hyperglycemia and ketosis, reported to be as high as 77.8% [37]. Furthermore, insulin should be strongly considered in those with previous T2DM who fail to control their diabetes with ICI treatments, given the risk of a new underlying insulin deficiency. In patients with T2DM already taking non-insulin therapies, the initiation of a long acting basal insulin and rapid acting prandial insulin should be strongly considered, as simply adjusting their current medical therapy for T2DM may be ineffective as insulin-resistance may no longer be the main driving force for their hyperglycemia (Table 3). Insulin therapy would be necessary to reduce their risk of acute DKA in these cases[1].

CONCLUSION

Patient education regarding symptoms of hyperglycemia is an important safety component when initiating any of these medications, as are the more critical symptoms of hyperventilation and nausea or vomiting that may be associated with imminent DKA upon ICI therapy, in particular. The appearance of these symptoms should trigger an immediate evaluation for hyperglycemia, endogenous insulin levels (post-meal C-peptide and insulin), and acidosis/ketones to rule out developing DKA.

Given the consequences of uncontrolled blood sugars for these patients, it is important to recognize and manage hyperglycemia during cancer therapy, whether because of a worsening control of pre-existing diabetes or new onset hyperglycemia arising as a side effect of the chemotherapy itself. Current recommendations suggest tailoring glycemic control according to the underlying etiology of the hyperglycemia (insulin-resistance *vs* insulin-deficiency)[16] (Table 3) and to also consider that many cancer therapies are prescribed in cycles, which will require monitoring and perhaps intermittent treatment of the hyperglycemia[106].

REFERENCES

1 **Stelmachowska-Banaś M**, Czajka-Oraniec I. Management of endocrine immune-related adverse events of immune checkpoint inhibitors: an updated review. *Endocr Connect* 2020; **9**: R207-R228 [PMID: 33064663 DOI: 10.1530/EC-20-0342]

2 **Bouillet B**, Buffier P, Smati S, Archambeaud F, Cariou B, Vergès B. Expert opinion on the metabolic complications of mTOR inhibitors. *Ann Endocrinol (Paris)* 2018; **79**: 583-590 [PMID: 30144939 DOI: 10.1016/j.ando.2018.07.010]

3 **Feng JP**, Yuan XL, Li M, Fang J, Xie T, Zhou Y, Zhu YM, Luo M, Lin M, Ye DW. Secondary diabetes associated with 5-fluorouracil-based chemotherapy regimens in non-diabetic patients with colorectal cancer: results from a single-centre cohort study. *Colorectal Dis* 2013; **15**: 27-33 [PMID: 22594556 DOI: 10.1111/j.1463-1318.2012.03097.x]

4 **Suh S**, Park MK. Glucocorticoid-Induced Diabetes Mellitus: An Important but Overlooked Problem. *Endocrinol Metab (Seoul)* 2017; **32**: 180-189 [PMID: 28555464 DOI: 10.3803/EnM.2017.32.2.180]

5 **Masrur S**, Cox M, Bhatt DL, Smith EE, Ellrodt G, Fonarow GC, Schwamm L. Association of Acute and Chronic Hyperglycemia With Acute Ischemic Stroke Outcomes Post-Thrombolysis: Findings From Get With The Guidelines-Stroke. *J Am Heart Assoc* 2015; **4**: e002193 [PMID: 26408015 DOI: 10.1161/JAHA.115.002193]

6 **Egi M**, Bellomo R, Stachowski E, French CJ, Hart GK, Hegarty C, Bailey M. Blood glucose concentration and outcome of critical illness: the impact of diabetes. *Crit Care Med* 2008; **36**: 2249-2255 [PMID: 18664780 DOI: 10.1097/CCM.0b013e318181039a]

7 **Hermanides J**, Vriesendorp TM, Bosman RJ, Zandstra DF, Hoekstra JB, Devries JH. Glucose variability is associated with intensive care unit mortality. *Crit Care Med* 2010; **38**: 838-842 [PMID: 20035218 DOI: 10.1097/CCM.0b013e3181cc4be9]

8 **Masur K**, Vetter C, Hinz A, Tomas N, Henrich H, Niggemann B, Zänker KS. Diabetogenic glucose and insulin concentrations modulate transcriptome and protein levels involved in tumour cell migration, adhesion and proliferation. *Br J Cancer* 2011; **104**: 345-352 [PMID: 21179032 DOI: 10.1038/sj.bjc.6606050]

9 **Gerards MC**, van der Velden DL, Baars JW, Brandjes DPM, Hoekstra JBL, Vriesendorp TM, Gerdes VEA. Impact of hyperglycemia on the efficacy of chemotherapy-A systematic review of preclinical studies. *Crit Rev Oncol Hematol* 2017; **113**: 235-241 [PMID: 28427512 DOI: 10.1016/j.critrevonc.2017.03.007]

10 **Matias Cdo N**, Lima V, Teixeira HM, Souto FR, Magalhães V. Hyperglycemia increases the complicated infection and mortality rates during induction therapy in adult acute leukemia patients. *Rev Bras Hematol Hemoter* 2013; **35**: 39-43 [PMID: 23580883 DOI: 10.5581/1516-8484.20130013]

11 **Healy SJ**, Nagaraja HN, Alwan D, Dungan KM. Prevalence, predictors, and outcomes of steroid-induced hyperglycemia in hospitalized patients with hematologic malignancies. *Endocrine* 2017; **56**: 90-97 [PMID: 28058528 DOI: 10.1007/s12020-016-1220-2]

12 **Jung SH**, Jang HC, Lee SS, Ahn JS, Yang DH, Kim YK, Kim HJ, Lee JJ. The impact of hyperglycemia on risk of severe infections during early period of induction therapy in patients with newly diagnosed multiple myeloma. *Biomed Res Int* 2014; **2014**: 413149 [PMID: 24822205 DOI: 10.1155/2014/413149]

13 **Ma YS**, Yang IP, Tsai HL, Huang CW, Juo SH, Wang JY. High glucose modulates antiproliferative effect and cytotoxicity of 5-fluorouracil in human colon cancer cells. *DNA Cell Biol* 2014; **33**: 64-72 [PMID: 24283362 DOI: 10.1089/dna.2013.2161]

14 **Perner A**, Nielsen SE, Rask-Madsen J. High glucose impairs superoxide production from isolated blood neutrophils. *Intensive Care Med* 2003; **29**: 642-645 [PMID: 12552364 DOI: 10.1007/s00134-002-1628-4]

15 **Brunello A**, Kapoor R, Extermann M. Hyperglycemia during chemotherapy for hematologic and solid tumors is correlated with increased toxicity. *Am J Clin Oncol* 2011; **34**: 292-296 [PMID: 20622641 DOI: 10.1097/COC.0b013e3181e1d0c0]

16 **Chowdhury TA**, Jacob P. Challenges in the management of people with diabetes and cancer. *Diabet Med* 2019; **36**: 795-802 [PMID: 30706527 DOI: 10.1111/dme.13919]

17 **Yang J**, Jia B, Qiao Y, Chen W, Qi X. Variations of blood glucose in cancer patients during chemotherapy. *Niger J Clin Pract* 2016; **19**: 704-708 [PMID: 27811438 DOI: 10.4103/1119-3077.187323]

18 **Brahmer JR**, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, Chau I, Ernstoff MS, Gardner JM, Ginex P, Hallmeyer S, Holter Chakrabarty J, Leighl NB, Mammen JS, McDermott DF, Naing A, Nastoupil LJ, Phillips T, Porter LD, Puzanov I, Reichner CA, Santomasso BD, Seigel C, Spira A, Suarez-Almazor ME, Wang Y, Weber JS, Wolchok JD, Thompson JA; National Comprehensive Cancer Network. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2018; **36**: 1714-1768 [PMID: 29442540 DOI: 10.1200/JCO.2017.77.6385]

19 **Robert C**. A decade of immune-checkpoint inhibitors in cancer therapy. *Nat Commun* 2020; **11**: 3801 [PMID: 32732879 DOI: 10.1038/s41467-020-17670-y]

20 **Himmel ME**, Saibil SD, Saltman AP. Immune checkpoint inhibitors in cancer immunotherapy. *CMAJ* 2020; **192**: E651 [PMID: 32540906 DOI: 10.1503/cmaj.191231]

21 **Puzanov I**, Ribas A, Robert C, Schachter J, Nyakas M, Daud A, Arance A, Carlino MS, O'Day SJ, Long GV, Margolin KA, Dummer R, Schadendorf D, Lutzky J, Ascierto PA, Tarhini A, Lin J, Mogg R, Homet Moreno B, Ibrahim N, Hamid O. Association of BRAF V600E/K Mutation Status and Prior BRAF/MEK Inhibition With Pembrolizumab Outcomes in Advanced Melanoma: Pooled Analysis of 3 Clinical Trials. *JAMA Oncol* 2020; **6**: 1256-1264 [PMID: 32672795 DOI: 10.1001/jamaoncol.2020.2288]

22 **Zhu AX**, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Verslype C, Zagonel V, Fartoux L, Vogel A, Sarker D, Verset G, Chan SL, Knox J, Daniele B, Webber AL, Ebbinghaus SW, Ma J, Siegel AB, Cheng AL, Kudo M; KEYNOTE-224 investigators. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018; **19**: 940-952 [PMID: 29875066 DOI: 10.1016/S1470-2045(18)30351-6]

23 **Finn RS**, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, Yau T, Garrido M, Chan SL, Knox J, Daniele B, Ebbinghaus SW, Chen E, Siegel AB, Zhu AX, Cheng AL; KEYNOTE-240 investigators. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. *J Clin Oncol* 2020; **38**: 193-202 [PMID: 31790344 DOI: 10.1200/JCO.19.01307]

24 **Hellmann MD**, Paz-Ares L, Bernabe Caro R, Zurawski B, Kim SW, Carcereny Costa E, Park K, Alexandru A, Lupinacci L, de la Mora Jimenez E, Sakai H, Albert I, Vergnenegre A, Peters S, Syrigos K, Barlesi F, Reck M, Borghaei H, Brahmer JR, O'Byrne KJ, Geese WJ, Bhagavatheeswaran P, Rabindran SK, Kasinathan RS, Nathan FE, Ramalingam SS. Nivolumab plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* 2019; **381**: 2020-2031 [PMID: 31562796 DOI: 10.1056/NEJMoa1910231]

25 **Motzer RJ**, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, Plimack ER, Barthélémy P, Porta C, George S, Powles T, Donskov F, Neiman V, Kollmannsberger CK, Salman P, Gurney H, Hawkins R, Ravaud A, Grimm MO, Bracarda S, Barrios CH, Tomita Y, Castellano D, Rini BI, Chen AC, Mekan S, McHenry MB, Wind-Rotolo M, Doan J, Sharma P, Hammers HJ, Escudier B; CheckMate 214 Investigators. Nivolumab plus Ipilimumab *vs* Sunitinib in Advanced Renal-Cell Carcinoma. *N Engl J Med* 2018; **378**: 1277-1290 [PMID: 29562145 DOI: 10.1056/NEJMoa1712126]

26 **Powles T**, Albiges L, Staehler M, Bensalah K, Dabestani S, Giles RH, Hofmann F, Hora M, Kuczyk MA, Lam TB, Marconi L, Merseburger AS, Fernández-Pello S, Tahbaz R, Volpe A, Ljungberg B, Bex A. Updated European Association of Urology Guidelines: Recommendations for the Treatment of First-line Metastatic Clear Cell Renal Cancer. *Eur Urol* 2018; **73**: 311-315 [PMID: 29223605 DOI: 10.1016/j.eururo.2017.11.016]

27 **Chang LS**, Barroso-Sousa R, Tolaney SM, Hodi FS, Kaiser UB, Min L. Endocrine Toxicity of Cancer Immunotherapy Targeting Immune Checkpoints. *Endocr Rev* 2019; **40**: 17-65 [PMID: 30184160 DOI: 10.1210/er.2018-00006]

28 **Stamatouli AM**, Quandt Z, Perdigoto AL, Clark PL, Kluger H, Weiss SA, Gettinger S, Sznol M, Young A, Rushakoff R, Lee J, Bluestone JA, Anderson M, Herold KC. Collateral Damage: Insulin-Dependent Diabetes Induced With Checkpoint Inhibitors. *Diabetes* 2018; **67**: 1471-1480 [PMID: 29937434 DOI: 10.2337/dbi18-0002]

29 **Chen L**, Han X. Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. *J Clin Invest* 2015; **125**: 3384-3391 [PMID: 26325035 DOI: 10.1172/JCI80011]

30 **Pauken KE**, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol* 2015; **36**: 265-276 [PMID: 25797516 DOI: 10.1016/j.it.2015.02.008]

31 **Kyi C**, Postow MA. Checkpoint blocking antibodies in cancer immunotherapy. *FEBS Lett* 2014; **588**: 368-376 [PMID: 24161671 DOI: 10.1016/j.febslet.2013.10.015]

32 **Bour-Jordan H**, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone JA. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. *Immunol Rev* 2011; **241**: 180-205 [PMID: 21488898 DOI: 10.1111/j.1600-065X.2011.01011.x]

33 **Tivol EA**, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 Leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995; **3**: 541-547 [PMID: 7584144 DOI: 10.1016/1074-7613(95)90125-6]

34 **Nogueira E**, Newsom-Davis T, Morganstein DL. Immunotherapy-induced endocrinopathies: assessment, management and monitoring. *Ther Adv Endocrinol Metab* 2019; **10**: 2042018819896182 [PMID: 31903179 DOI: 10.1177/2042018819896182]

35 **Barrett JC**, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS; Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 Loci affect risk of type 1 diabetes. *Nat Genet* 2009; **41**: 703-707 [PMID: 19430480 DOI: 10.1038/ng.381]

36 **van Lummel M**, van Veelen PA, de Ru AH, Janssen GM, Pool J, Laban S, Joosten AM, Nikolic T, Drijfhout JW, Mearin ML, Aanstoot HJ, Peakman M, Roep BO. Dendritic Cells Guide Islet Autoimmunity through a Restricted and Uniquely Processed Peptidome Presented by High-Risk HLA-DR. *J Immunol* 2016; **196**: 3253-3263 [PMID: 26944932 DOI: 10.4049/jimmunol.1501282]

37 **Kyriacou A**, Melson E, Chen W, Kempegowda P. Is immune checkpoint inhibitor-associated diabetes the same as fulminant type 1 diabetes mellitus? *Clin Med (Lond)* 2020; **20**: 417-423 [PMID: 32675150 DOI: 10.7861/clinmed.2020-0054]

38 **Shieh SJ**, Chou FC, Yu PN, Lin WC, Chang DM, Roffler SR, Sytwu HK. Transgenic expression of single-chain anti-CTLA-4 Fv on beta cells protects nonobese diabetic mice from autoimmune diabetes. *J Immunol* 2009; **183**: 2277-2285 [PMID: 19635924 DOI: 10.4049/jimmunol.0900679]

39 **Clotman K**, Janssens K, Specenier P, Weets I, De Block CEM. Programmed Cell Death-1 Inhibitor-Induced Type 1 Diabetes Mellitus. *J Clin Endocrinol Metab* 2018; **103**: 3144-3154 [PMID: 29955867 DOI: 10.1210/jc.2018-00728]

40 **Kotwal A**, Haddox C, Block M, Kudva YC. Immune checkpoint inhibitors: an emerging cause of insulin-dependent diabetes. *BMJ Open Diabetes Res Care* 2019; **7**: e000591 [PMID: 30899528 DOI: 10.1136/bmjdrc-2018-000591]

41 **Chae YK**, Chiec L, Mohindra N, Gentzler R, Patel J, Giles F. A case of pembrolizumab-induced type-1 diabetes mellitus and discussion of immune checkpoint inhibitor-induced type 1 diabetes. *Cancer Immunol Immunother* 2017; **66**: 25-32 [PMID: 27761609 DOI: 10.1007/s00262-016-1913-7]

42 **Aleksova J**, Lau PK, Soldatos G, McArthur G. Glucocorticoids did not reverse type 1 diabetes mellitus secondary to pembrolizumab in a patient with metastatic melanoma. *BMJ Case Rep* 2016; **2016** [PMID: 27881588 DOI: 10.1136/bcr-2016-217454]

43 **Trinh B**, Donath MY, Läubli H. Successful Treatment of Immune Checkpoint Inhibitor-Induced Diabetes With Infliximab. *Diabetes Care* 2019; **42**: e153-e154 [PMID: 31308021 DOI: 10.2337/dc19-0908]

44 **Hong AR**, Yoon JH, Kim HK, Kang HC. Immune Checkpoint Inhibitor-Induced Diabetic Ketoacidosis: A Report of Four Cases and Literature Review. *Front Endocrinol (Lausanne)* 2020; **11**: 14 [PMID: 32047478 DOI: 10.3389/fendo.2020.00014]

45 **Imagawa A**, Hanafusa T, Miyagawa J, Matsuzawa Y. A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. Osaka IDDM Study Group. *N Engl J Med* 2000; **342**: 301-307 [PMID: 10655528 DOI: 10.1056/NEJM200002033420501]

46 **Condon KJ**, Sabatini DM. Nutrient regulation of mTORC1 at a glance. *J Cell Sci* 2019; **132** [PMID: 31722960 DOI: 10.1242/jcs.222570]

47 **Polivka J Jr**, Janku F. Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. *Pharmacol Ther* 2014; **142**: 164-175 [PMID: 24333502 DOI: 10.1016/j.pharmthera.2013.12.004]

48 **Yu JS**, Cui W. Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. *Development* 2016; **143**: 3050-3060 [PMID: 27578176 DOI: 10.1242/dev.137075]

49 **Porta C**, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR Signaling in Cancer. *Front Oncol* 2014; **4**: 64 [PMID: 24782981 DOI: 10.3389/fonc.2014.00064]

50 **Bastidas RJ**, Shertz CA, Lee SC, Heitman J, Cardenas ME. Rapamycin exerts antifungal activity *in vitro* and *in vivo* against Mucor circinelloides *via* FKBP12-dependent inhibition of Tor. *Eukaryot Cell* 2012; **11**: 270-281 [PMID: 22210828 DOI: 10.1128/EC.05284-11]

51 **Cloughesy TF**, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, Hsueh T, Chen Y, Wang W, Youngkin D, Liau L, Martin N, Becker D, Bergsneider M, Lai A, Green R, Oglesby T, Koleto M, Trent J, Horvath S, Mischel PS, Mellinghoff IK, Sawyers CL. Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. *PLoS Med* 2008; **5**: e8 [PMID: 18215105 DOI: 10.1371/journal.pmed.0050008]

52 **Tian T**, Li X, Zhang J. mTOR Signaling in Cancer and mTOR Inhibitors in Solid Tumor Targeting Therapy. *Int J Mol Sci* 2019; **20**: 755 [PMID: 30754640 DOI: 10.3390/ijms20030755]

53 **Yang J**, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Mol Cancer* 2019; **18**: 26 [PMID: 30782187 DOI: 10.1186/s12943-019-0954-x]

54 **Gu Y**, Lindner J, Kumar A, Yuan W, Magnuson MA. Rictor/mTORC2 is essential for maintaining a balance between beta-cell proliferation and cell size. *Diabetes* 2011; **60**: 827-837 [PMID: 21266327 DOI: 10.2337/db10-1194]

55 **Fraenkel M**, Ketzinel-Gilad M, Ariav Y, Pappo O, Karaca M, Castel J, Berthault MF, Magnan C, Cerasi E, Kaiser N, Leibowitz G. mTOR inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia and exacerbates the metabolic state in type 2 diabetes. *Diabetes* 2008; **57**: 945-957 [PMID: 18174523 DOI: 10.2337/db07-0922]

56 **Laplante M**, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**: 274-293 [PMID: 22500797 DOI: 10.1016/j.cell.2012.03.017]

57 **Kakiuchi Y**, Yurube T, Kakutani K, Takada T, Ito M, Takeoka Y, Kanda Y, Miyazaki S, Kuroda R, Nishida K. Pharmacological inhibition of mTORC1 but not mTORC2 protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism through Akt and autophagy induction. *Osteoarthritis Cartilage* 2019; **27**: 965-976 [PMID: 30716534 DOI: 10.1016/j.joca.2019.01.009]

58 **Sivendran S**, Agarwal N, Gartrell B, Ying J, Boucher KM, Choueiri TK, Sonpavde G, Oh WK, Galsky MD. Metabolic complications with the use of mTOR inhibitors for cancer therapy. *Cancer Treat Rev* 2014; **40**: 190-196 [PMID: 23684373 DOI: 10.1016/j.ctrv.2013.04.005]

59 **Khan KH**, Wong M, Rihawi K, Bodla S, Morganstein D, Banerji U, Molife LR. Hyperglycemia and Phosphatidylinositol 3-Kinase/Protein Kinase B/Mammalian Target of Rapamycin (PI3K/AKT/mTOR) Inhibitors in Phase I Trials: Incidence, Predictive Factors, and Management. *Oncologist* 2016; **21**: 855-860 [PMID: 27151652 DOI: 10.1634/theoncologist.2015-0248]

60 **Zhang Y**, Yan H, Xu Z, Yang B, Luo P, He Q. Molecular basis for class side effects associated with PI3K/AKT/mTOR pathway inhibitors. *Expert Opin Drug Metab Toxicol* 2019; **15**: 767-774 [PMID: 31478386 DOI: 10.1080/17425255.2019.1663169]

61 **Crouthamel MC**, Kahana JA, Korenchuk S, Zhang SY, Sundaresan G, Eberwein DJ, Brown KK, Kumar R. Mechanism and management of AKT inhibitor-induced hyperglycemia. *Clin Cancer Res* 2009; **15**: 217-225 [PMID: 19118049 DOI: 10.1158/1078-0432.CCR-08-1253]

62 **Jebali M**, Elaidi R, Brizard M, Fouque J, Takouchop C, Sabatier B, Oudard S, Medioni J. Biological toxicities as surrogate markers of efficacy in patients treated with mTOR inhibitors for metastatic renal cell carcinoma. *BMC Cancer* 2017; **17**: 27 [PMID: 28061764 DOI: 10.1186/s12885-016-2993-7]

63 **Ma H**, Ren Z, Wang J, Shen X, Li L. mTOR Inhibitor Everolimus Causing Diabetic ketoacidosis and Acute Pancreatitis. *Int J Clin Case Stud* 2017; **3**: 120 [DOI: 10.15344/2455-2356/2017/120]

64 **Busaidy NL**, Farooki A, Dowlati A, Perentesis JP, Dancey JE, Doyle LA, Brell JM, Siu LL. Management of metabolic effects associated with anticancer agents targeting the PI3K-Akt-mTOR pathway. *J Clin Oncol* 2012; **30**: 2919-2928 [PMID: 22778315 DOI: 10.1200/JCO.2011.39.7356]

65 **Lee JJ**, Beumer JH, Chu E. Therapeutic drug monitoring of 5-fluorouracil. *Cancer Chemother Pharmacol* 2016; **78**: 447-464 [PMID: 27217046 DOI: 10.1007/s00280-016-3054-2]

66 **Walko CM**, Lindley C. Capecitabine: a review. *Clin Ther* 2005; **27**: 23-44 [PMID: 15763604 DOI: 10.1016/j.clinthera.2005.01.005]

67 **Zhang N**, Yin Y, Xu SJ, Chen WS. 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules* 2008; **13**: 1551-1569 [PMID: 18794772 DOI: 10.3390/molecules13081551]

68 **Fujii S**, Ikenaka K, Fukushima M, Shirasaka T. Effect of uracil and its derivatives on antitumor activity of 5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil. *Gan* 1978; **69**: 763-772 [PMID: 750271]

69 **Taguchi T**. Experience with UFT in Japan. *Oncology (Williston Park)* 1997; **11**: 30-34 [PMID: 9348564]

70 **Hashimoto Y**, Yoshida Y, Yamada T, Aisu N, Yoshimatsu G, Yoshimura F, Hasegawa S. Current Status of Therapeutic Drug Monitoring of 5-Fluorouracil Prodrugs. *Anticancer Res* 2020; **40**: 4655-4661 [PMID: 32727789 DOI: 10.21873/anticanres.14464]

71 **Avishek A**, Jayanthi M, Biswajit D. Capecitabine-induced hyperglycemia without hyperlipidemia: a case report. *Eur J Clin Pharmacol* 2017; **73**: 1519-1521 [PMID: 28752256 DOI: 10.1007/s00228-017-2304-5]

72 **Adachi J**, Mimura M, Gotyo N, Watanabe T. The development of fulminant type 1 diabetes during chemotherapy for rectal cancer. *Intern Med* 2015; **54**: 819-822 [PMID: 25832949 DOI: 10.2169/internalmedicine.54.3413]

73 **Iwata Y**, Matsuhashi N, Takahashi T, Suetsugu T, Fukada M, Yasufuku I, Imai T, Tanahashi T, Matsui S, Imai H, Tanaka Y, Yamaguchi K, Yoshida K. Diabetic ketoacidosis caused by fulminant type 1 diabetes during adjuvant chemotherapy for colon cancer: A case report. *Mol Clin Oncol* 2019; **11**: 189-191 [PMID: 31281655 DOI: 10.3892/mco.2019.1862]

74 **Tayek JA**, Chlebowski RT. Metabolic response to chemotherapy in colon cancer patients. *JPEN J Parenter Enteral Nutr* 1992; **16**: 65S-71S [PMID: 1287227 DOI: 10.1177/014860719201600606]

75 **Feng JP**, Chen JG, Yuan XL, Wang YP, Fang J, Liu C. [Impact of 5-fluorouracil on glucose metabolism and pancreatic pathology in rats]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2010; **13**: 935-938 [PMID: 21186417]

76 **Komatsu T**, Yamazaki H, Shimada N, Nagayama S, Kawaguchi Y, Nakajima M, Yokoi T. Involvement of microsomal cytochrome P450 and cytosolic thymidine phosphorylase in 5-fluorouracil formation from tegafur in human liver. *Clin Cancer Res* 2001; **7**: 675-681 [PMID: 11297264]

77 **Yucel H**, Warmerdam LV. Capecitabine-induced pancreatitis. *J Oncol Pharm Pract* 2010; **16**: 133-134 [PMID: 19700478 DOI: 10.1177/1078155209344650]

78 **Jones KL**, Valero V. Capecitabine-induced pancreatitis. *Pharmacotherapy* 2003; **23**: 1076-1078 [PMID: 12921254 DOI: 10.1592/phco.23.8.1076.32870]

79 **Duman BB**, Paydas S, Tetiker T, Gunaldı M, Afsar CU, Erçolak V, Haksöyler V, Dilli MŞ. Capecitabine-induced hypertriglyceridemia and hyperglycemia: two cases. *Pharmacology* 2012; **90**: 212-215 [PMID: 23038659 DOI: 10.1159/000342382]

80 **Han GH**, Huang JX. Hypertriglyceridemia and hyperglycemia induced by capecitabine: a report of two cases and review of the literature. *J Oncol Pharm Pract* 2015; **21**: 380-383 [PMID: 24781450 DOI: 10.1177/1078155214532508]

81 **Nogué M**, Rambaud J, Fabre S, Filippi N, Jorgensen C, Pers YM. Long-term corticosteroid use and dietary advice: a qualitative analysis of the difficulties encountered by patient. *BMC Health Serv Res* 2019; **19**: 255 [PMID: 31027493 DOI: 10.1186/s12913-019-4052-y]

82 **Pantelidis P**, Tsitsopoulos PP, Pappa E, Theologou E, Karanikolas N, Drosos C, Tsonidis C. The effect of diabetes mellitus on in-hospital hyperglycemia, length of stay and survival in patients with brain tumor receiving dexamethasone: A descriptive and comparative analysis. *Clin Neurol Neurosurg* 2019; **184**: 105450 [PMID: 31376773 DOI: 10.1016/j.clineuro.2019.105450]

83 **Van Ryckeghem F**. Corticosteroids, the oldest agent in the prevention of chemotherapy-induced nausea and vomiting: What about the guidelines? *J Transl Int Med* 2016; **4**: 46-51 [PMID: 28191518 DOI: 10.1515/jtim-2016-0010]

84 **Lossignol D**. A little help from steroids in oncology. *J Transl Int Med* 2016; **4**: 52-54 [PMID: 28191519 DOI: 10.1515/jtim-2016-0011]

85 **Lyall MJ**, Thethy I, Steven L, MacKean M, Nussey F, Sakala M, Rye T, Strachan MWJ, Dover AR. Diurnal profile of interstitial glucose following dexamethasone prophylaxis for chemotherapy treatment of gynaecological cancer. *Diabet Med* 2018; **35**: 1508-1514 [PMID: 29938852 DOI: 10.1111/dme.13770]

86 **Zylla D**, Gilmore G, Eklund J, Richter S, Carlson A. Impact of diabetes and hyperglycemia on health care utilization, infection risk, and survival in patients with cancer receiving glucocorticoids with chemotherapy. *J Diabetes Complications* 2019; **33**: 335-339 [PMID: 30717892 DOI: 10.1016/j.jdiacomp.2018.12.012]

87 **Donihi AC**, Raval D, Saul M, Korytkowski MT, DeVita MA. Prevalence and predictors of

corticosteroid-related hyperglycemia in hospitalized patients. *Endocr Pract* 2006; **12**: 358-362 [PMID: 16901792 DOI: 10.4158/EP.12.4.358]

88 **Sonabend RY**, McKay SV, Okcu MF, Yan J, Haymond MW, Margolin JF. Hyperglycemia during induction therapy is associated with poorer survival in children with acute lymphocytic leukemia. *J Pediatr* 2009; **155**: 73-78 [PMID: 19394046 DOI: 10.1016/j.jpeds.2009.01.072]

89 **Weiser MA**, Cabanillas ME, Konopleva M, Thomas DA, Pierce SA, Escalante CP, Kantarjian HM, O'Brien SM. Relation between the duration of remission and hyperglycemia during induction chemotherapy for acute lymphocytic leukemia with a hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone/methotrexate-cytarabine regimen. *Cancer* 2004; **100**: 1179-1185 [PMID: 15022284 DOI: 10.1002/cncr.20071]

90 **Flory J**, Farooki A. Diabetes Management in Cancer Patients. *Oncology (Williston Park)* 2016; **30**: 565-570 [PMID: 27306711]

91 **Burt MG**, Drake SM, Aguilar-Loza NR, Esterman A, Stranks SN, Roberts GW. Efficacy of a basal bolus insulin protocol to treat prednisolone-induced hyperglycaemia in hospitalised patients. *Intern Med J* 2015; **45**: 261-266 [PMID: 25565560 DOI: 10.1111/imj.12680]

92 **Grimes A**, Mohamed A, Sopfe J, Hill R, Lynch J. Hyperglycemia During Childhood Cancer Therapy: Incidence, Implications, and Impact on Outcomes. *J Natl Cancer Inst Monogr* 2019; **2019**: 132-138 [PMID: 31532529 DOI: 10.1093/jncimonographs/Lgz022]

93 **Aisyi M**, Andriastuti M, Kurniati N. The Effect of Combination of Steroid and L-Asparaginase on Hyperglycemia in Children with Acute Lymphoblastic Leukemia (ALL). *Asian Pac J Cancer Prev* 2019; **20**: 2619-2624 [PMID: 31554355 DOI: 10.31557/APJCP.2019.20.9.2619]

94 **Gosmanov AR**, Goorha S, Stelts S, Peng L, Umpierrez GE. Management of hyperglycemia in diabetic patients with hematologic malignancies during dexamethasone therapy. *Endocr Pract* 2013; **19**: 231-235 [PMID: 23337144 DOI: 10.4158/EP12256.OR]

95 **Ochola LA**, Nyamu DG, Guantai EM, Weru IW. Metformin's effectiveness in preventing prednisone-induced hyperglycemia in hematological cancers. *J Oncol Pharm Pract* 2020; **26**: 823-834 [PMID: 31495292 DOI: 10.1177/1078155219873048]

96 **Rowbottom L**, Stinson J, McDonald R, Emmenegger U, Cheng S, Lowe J, Giotis A, Cheon P, Chow R, Pasetka M, Thavarajah N, Pulenzas N, Chow E, DeAngelis C. Retrospective review of the incidence of monitoring blood glucose levels in patients receiving corticosteroids with systemic anticancer therapy. *Ann Palliat Med* 2015; **4**: 70-77 [PMID: 25971294 DOI: 10.3978/j.issn.2224-5820.2015.04.07]

97 **Storey S**, Von Ah D. Impact of malglycemia on clinical outcomes in hospitalized patients with cancer: a review of the literature. *Oncol Nurs Forum* 2012; **39**: 458-465 [PMID: 22940510 DOI: 10.1188/12.ONF.458-465]

98 **Yoo KE**, Kang RY, Lee JY, Lee YJ, Suh SY, Kim KS, Kim HS, Lee SH, Lee BK. Awareness of the adverse effects associated with prophylactic corticosteroid use during docetaxel therapy. *Support Care Cancer* 2015; **23**: 1969-1977 [PMID: 25500718 DOI: 10.1007/s00520-014-2547-y]

99 **Yuen KC**, McDaniel PA, Riddle MC. Twenty-four-hour profiles of plasma glucose, insulin, C-peptide and free fatty acid in subjects with varying degrees of glucose tolerance following short-term, medium-dose prednisone (20 mg/day) treatment: evidence for differing effects on insulin secretion and action. *Clin Endocrinol (Oxf)* 2012; **77**: 224-232 [PMID: 21973241 DOI: 10.1111/j.1365-2265.2011.04242.x]

100 **Tamez-Pérez HE**, Quintanilla-Flores DL, Rodríguez-Gutiérrez R, González-González JG, Tamez-Peña AL. Steroid hyperglycemia: Prevalence, early detection and therapeutic recommendations: A narrative review. *World J Diabetes* 2015; **6**: 1073-1081 [PMID: 26240704 DOI: 10.4239/wjd.v6.i8.1073]

101 **Clore JN**, Thurby-Hay L. Glucocorticoid-induced hyperglycemia. *Endocr Pract* 2009; **15**: 469-474 [PMID: 19454391 DOI: 10.4158/EP08331.RAR]

102 **Lamar ZS**, Dothard A, Kennedy L, Isom S, Robinson M, Vaidya R, Hurd D, McClain D, Lesser G. Hyperglycemia during first-line R-CHOP or dose adjusted R-EPOCH chemotherapy for non-Hodgkin lymphoma is prevalent and associated with chemotherapy alteration - a retrospective study. *Leuk Lymphoma* 2018; **59**: 1871-1877 [PMID: 29252084 DOI: 10.1080/10428194.2017.1410889]

103 **Kwon S**, Hermayer KL, Hermayer K. Glucocorticoid-induced hyperglycemia. *Am J Med Sci* 2013; **345**: 274-277 [PMID: 23531958 DOI: 10.1097/MAJ.0b013e31828a6a01]

104 **Yates CJ**, Fourlanos S, Colman PG, Cohney SJ. Divided dosing reduces prednisolone-induced hyperglycaemia and glycaemic variability: a randomized trial after kidney transplantation. *Nephrol Dial Transplant* 2014; **29**: 698-705 [PMID: 24009292 DOI: 10.1093/ndt/gft377]

105 **Brady V**, Thosani S, Zhou S, Bassett R, Busaidy NL, Lavis V. Safe and effective dosing of basal-bolus insulin in patients receiving high-dose steroids for hyper-cyclophosphamide, doxorubicin, vincristine, and dexamethasone chemotherapy. *Diabetes Technol Ther* 2014; **16**: 874-879 [PMID: 25321387 DOI: 10.1089/dia.2014.0115]

106 **Jacob P**, Chowdhury TA. Management of diabetes in patients with cancer. *QJM* 2015; **108**: 443-448 [PMID: 25362096 DOI: 10.1093/qjmed/hcu218]

107 **Mobasseri M**, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. *Health Promot Perspect* 2020; **10**: 98-115 [PMID: 32296622 DOI: 10.34172/hpp.2020.18]

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World J Diabetes 2021 July 15; 12(7): 1026-1041

DOI: 10.4239/wjd.v12.i7.1026

ISSN 1948-9358 (online)

REVIEW

Immunometabolic bases of type 2 diabetes in the severity of COVID-19

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Author contributions: Viurcos-Sanabria R and Escobedo G read the literature and wrote the manuscript; all authors have read and approved the final manuscript.

Supported by the Fondo Sectorial de Investigación para la Educación-CONACYT-México, No. CB-2016-01-286209; and the Fondo Sectorial de Investigación y Desarrollo en Salud y Seguridad Social SS/IMSS/ISSSTE/CONACYT-México, No. SALUD-2017-02-290345.

Conflict-of-interest statement: The authors declare that there is no conflict of interest.

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Abstract

The outbreak of coronavirus disease 2019 (COVID-19) is caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). COVID-19 and type 2 diabetes (T2D) have now merged into an ongoing global syndemic that is threatening the lives of millions of people around the globe. For this reason, there is a deep need to understand the immunometabolic bases of the main etiological factors of T2D that affect the severity of COVID-19. Here, we discuss how hyperglycemia contributes to the cytokine storm commonly associated with COVID-19 by stimulating monocytes and macrophages to produce interleukin IL-1β, IL-6, and TNF-α in the airway epithelium. The main mechanisms through which hyperglycemia promotes reactive oxygen species release, inhibition of T cell activation, and neutrophil extracellular traps in the lungs of patients with severe SARS-CoV-2 infection are also studied. We further examine the molecular mechanisms by which proinflammatory cytokines induce insulin resistance, and their deleterious effects on pancreatic β-cell exhaustion in T2D patients critically ill with COVID-19. We address the effect of excess glucose on advanced glycation end product (AGE) formation and the role of AGEs in perpetuating pneumonia and acute respiratory distress syndrome. Finally, we discuss the contribution of preexisting endothelial dysfunction secondary to diabetes in the development of neutrophil trafficking, vascular leaking, and thrombotic events in patients with severe SARS-CoV-2 infection. As we outline here, T2D acts in synergy with SARS-CoV-2 infection to increase the progression, severity, and mortality of COVID-19. We think a better understanding of the T2D-related immunometabolic factors that contribute to exacerbate the severity of COVID-19 will improve our ability to identify patients with high mortality risk and prevent adverse outcomes.

Key Words: COVID-19; SARS-CoV-2; Type 2 diabetes; Inflammation; Hyperglycemia; Prothrombotic state

Manuscript source: Invited manuscript

Specialty type: Immunology

Country/Territory of origin: Mexico

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 3, 2021
Peer-review started: February 3, 2021
First decision: March 1, 2021
Revised: April 16, 2021
Accepted: June 16, 2021
Article in press: June 16, 2021
Published online: July 15, 2021

P-Reviewer: Khan MKA
S-Editor: Zhang H
L-Editor: A
P-Editor: Wang LL

Core Tip: Type 2 diabetes (T2D) acts in synergy with severe acute respiratory syndrome coronavirus-2 infection to increase the progression, severity, and mortality of coronavirus disease 2019 (COVID-19). Thus, the immunometabolic bases of the main etiological factors of T2D that contribute to the severity of COVID-19 should be studied. Here, we discuss the molecular mechanisms by which immune cells, hyperglycemia, hyperinsulinemia, loss of pancreatic β-cell mass, insulin resistance, advanced glycation end products, endothelial dysfunction, and prothrombotic state contribute to the severity of COVID-19. The syndemic between COVID-19 and T2D has challenged our ability to identify patients with high mortality risk based on scientific evidence.

Citation: Viurcos-Sanabria R, Escobedo G. Immunometabolic bases of type 2 diabetes in the severity of COVID-19. *World J Diabetes* 2021; 12(7): 1026-1041
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1026.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1026

INTRODUCTION

The novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first identified in the city of Wuhan, China in December 2019[1]. Unlike the SARS-CoV and the middle east respiratory syndrome coronavirus, SARS-CoV-2 is highly transmissible to humans, with case fatality rates ranging from 3% to 11%[2]. A study conducted in 1099 patients from China showed that incubation of SARS-CoV-2 takes a median of 4 d on average[3], while in the United States, the Centers for Disease Control and Prevention has estimated that symptoms can appear within 2-14 d after exposure[4]. The main modes of SARS-CoV-2 transmission are respiratory droplets produced by infected individuals, aerosols, and direct contact with contaminated surfaces or objects[5]. Transmission of SARS-CoV-2 is more likely to occur in the early stage of infection; by day 10 after the onset of symptoms, 90% of patients with mild disease show a negative RNA test[6,7]. SARS-CoV-2 is the causal agent of the coronavirus disease 2019 (COVID-19), an ongoing global pandemic that is affecting the lives of millions of people worldwide[8].

Although more than 85% of patients with COVID-19 experience a self-limiting illness with symptoms such as fever, headache, myalgia, and diarrhea, some patients develop the most severe forms of the disease including pneumonia, acute respiratory distress syndrome (ARDS), sepsis, multiple organ failure, and death[9]. For this reason, there is a deep need to understand the variety of factors that increase the severity of COVID-19.

The most severe and fatal cases of COVID-19 have been reported to occur in patients with preexisting comorbidities such as cancer, hypertension, and diabetes mellitus[10,11]. In fact, diabetes mellitus is one of the most prevalent comorbidities in patients critically ill with COVID-19[12]. Diabetes mellitus is a complex disorder characterized by abnormally high blood glucose levels that affect blood vessels and nerves. Diabetes ultimately results in chronic damage to skin, feet, immune system, eyes, kidneys, brain, and heart[13]. People living with diabetes do not exhibit increased susceptibility to SARS-CoV-2 infection compared to non-diabetic individuals [12]. However, patients with diabetes and COVID-19 are at much higher risk for adverse outcomes including admission to intensive care units, invasive ventilatory support, hospital-acquired infections, and death[14,15]. Diabetic patients with COVID-19 have a mortality rate of 7.3% with respect to non-diabetic subjects, among whom a mortality of 2.3% has been reported[14].

Type 2 diabetes (T2D), formerly known as adult-onset diabetes, is the most common form of diabetes and is characterized by multiple disorders including hyperglycemia, insulin resistance, systemic inflammation, hyperinsulinemia, β-cell exhaustion, vascular damage, endothelial dysfunction, nerve degeneration, increased platelet reactivity and prothrombotic state, and dyslipidemia, among others[16,17]. These conditions affect the immune response to pathogens through different mechanisms that are not yet fully understood and might influence the severity of COVID-19, as will

be further reviewed[16,18-23].

HYPERGLYCEMIA

Hyperglycemia is the most common metabolic alteration associated with T2D and is characterized by persistently high blood sugar levels[24]. Hyperglycemia and the immune response are known to affect each other in ways that directly impact the severity of the SARS-CoV-2 infection. It is well known that high glucose levels can activate multiple immune cell types, leading to enhanced production of proinflammatory cytokines such as interleukin IL-1 beta, IL-6, and TNF-α, among others[25]. This phenomenon is especially relevant to SARS-CoV-2 infection, because patients with T2D and COVID-19 exhibit increased serum levels of proinflammatory cytokines including IL-1β, IL-6, TNF-α, IL-8, and IFN-γ[26-30]. In patients with SARS-CoV-2 infection, this so-called "cytokine storm" is accompanied by other inflammation-related markers such as C-reactive protein, D-dimer, and ferritin, and linked to the severity of COVID-19[31]. Exacerbation of the inflammatory response causes a more severe acute infection that leads to ARDS and multiple organ failure[26]. Elevated levels of IL-6 have been found in both the airway epithelium and blood stream of patients with COVID-19 that develop severe ARDS[32].

Accumulating evidence confirms that hyperglycemia is a negative predictor in COVID-19 due to increased release of inflammatory mediators, endothelial dysfunction, thrombosis, and production of reactive oxygen species (ROS)[33-35]. Accordingly, T2D patients critically ill with COVID-19 that show acute hyperglycemic peaks at hospital admission have a worse prognosis than patients with glycated hemoglobin (HbA1c) levels near to 6.5%[36,37]. Therefore, a high blood glucose value at hospital admission is a risk factor for mortality in patients with severe SARS-CoV-2 infection[11,38].

Persistent hyperglycemia promotes mitochondrial oxidative stress and ROS production[39] that in turn leads to blood vessel damage, pancreatic beta cell dysfunction, and impaired insulin secretion[40,41]. Hyperglycemia-induced mitochondrial dysfunction triggers release of intracellular signaling molecules that can in turn inhibit the T cell response, a phenomenon that has been consistently reported in patients with severe COVID-19[42]. Moreover, SARS-CoV-2 can also directly induce mitochondrial ROS production by activating the HIF-1α that in turn is able to promote the proinflammatory cytokine storm[42,43]. Therefore, it is feasible that hyperglycemia and ROS production from T2D act in synergy with SARS-CoV-2 infection to aggravate the cellular damage, organ failure, and progression of COVID-19.

In parallel, high blood sugar levels stimulate lactate dehydrogenase (LDH) activity and increase lactate production[44]. This phenomenon is of particular interest in COVID-19, where it has been reported that increased LDH levels are accurate predictors of mortality in patients with severe SARS-CoV-2 infection[45]. Notably, increased lactate levels in T2D patients might delay clearance of SARS-CoV-2 by inhibiting the retinoic acid-inducible RIG-1-like receptor *via* the mitochondrial antiviral-signaling protein, which results in blockage of interferon production and reduced anti-viral response[44].

Likewise, natural killer (NK) cells are innate lymphocytes that eliminate virally infected cells. In T2D patients, hyperglycemia appears to increase NK cells that express low levels of the NKG2D and the natural cytotoxicity receptor NKp46, resulting in decreased degranulation capacity and inefficient anti-viral activity[46-48]. In addition, hyperglycemia promotes viral replication in monocytes with concomitant inhibition of T cell activation[42]. Some studies have also found low numbers of dendritic cells (DCs) in T2D patients with poor glycemic control. In line with this finding, high glucose conditions in *in vitro* cultures appears to prevent monocyte differentiation into DCs[49,50]. Moreover, DCs from T2D patients poorly induce T cell proliferation *in vitro*[51]. Taken together, all of this evidence supports a decisive role for hyperglycemia in exacerbating COVID-19 progression, since T2D patients with severe SARS-CoV-2 infection exhibit low cell counts of NK cells, functional DCs, and CD4+ and CD8+ T cells[52].

Hyperglycemia in T2D patients has deleterious effects on numerous neutrophil functions including migration, phagocytosis, and bacterial killing[53]. Additionally, T2D patients have low numbers of IFN-γ-producing cells, which affects viral clearance in multiple infections such as those provoked by cytomegalovirus, Epstein-Barr virus, and influenza[54]. Moreover, hyperglycemia reduces antibody titers and the ability to kill bacteria able to invade the lungs and cause pneumonia such as *Staphylococcus*

pneumoniae and *Staphylococcus aureus*[55]. Considering the immune effects of hyperglycemia within the context of SARS-CoV-2 infection, the mechanisms discussed above might partially explain why T2D patients are at a higher risk of developing severe COVID-19 and adverse outcomes including increased viral load, sepsis, and death. For this reason, uncontrolled hyperglycemia should be considered as a crucial risk factor for COVID-19 progression in T2D patients.

HYPERINSULINEMIA AND PANCREATIC B-CELL EXHAUSTION

Pancreatic β-cells play a key role in the control of blood glucose levels by secreting insulin[56]. During the evolution of T2D, pancreatic β-cells are exposed to glucotoxicity, ROS, and endoplasmic reticulum stress, all of which increase β-cell apoptosis and dysfunction[56,57]. Additionally, chronic hyperglycemia increases M1-like macrophage infiltration in pancreatic islets, where these immune cells can secrete IL-1β, IL-6, and TNF-α, promoting islet inflammation, β-cell malfunction, and apoptosis [58,59]. In response, pancreatic β-cells enhance insulin secretion in order to counteract persistently high glucose levels, leading to a state of hyperinsulinemia[60]. Nevertheless, β-cell mass is eventually exhausted, resulting in impaired insulin production. By the time diabetes is typically diagnosed, β-cells show less than fifty percent activity and are no longer able to secrete enough insulin to effectively maintain blood glucose levels[61,62].

In T2D patients, β-cell dysfunction can be aggravated during COVID-19 due to the ability of SARS-CoV-2 to enter the human pancreatic islets *via* angiotensin-converting enzyme 2 (ACE2)[62]. After SARS-CoV-2 invasion, inflammatory cells are recruited to pancreatic tissue, where they intensify local inflammation and injury resulting in increased peri- and intra islet fibrosis, β-cell mass loss, and hyperglycemia in both non-diabetic and diabetic patients[63]. Additionally, COVID-19 is characterized by persistent acute hypoxia that can affect numerous organs, including pancreatic islets, and provoke β-cell apoptosis directly[64]. In this way, SARS-CoV-2-induced pancreatic damage contributes to impaired insulin secretion that in turn may accelerate diabetes pathogenesis and/or aggravate preexisting diabetes[65]. Similarly, β-cell dysfunction that leads to chronic hyperglycemia is accompanied by ROS release, advanced glycation end product (AGE) formation, mitochondrial oxidative stress, and low antioxidant activity, worsening pancreatic β-cell damage during COVID-19[66-69].

A growing body of evidence suggests that SARS-CoV-2 infection not only affects the endocrine pancreas but also the exocrine pancreas[70]. In fact, SARS-CoV-2 appears to bind and enter the exocrine pancreatic ductal cells *via* ACE2[71]. Some studies estimate that the prevalence of the development of acute pancreatitis in patients with severe COVID-19 is as high as 17%[72].

The mechanisms discussed above highlight the importance of inflammation, hyperglycemia, and pancreatic dysfunction as potential contributors to the development of severe COVID-19 in patients with T2D[73].

INSULIN RESISTANCE

Insulin resistance is defined as the inability of insulin to exert its functions in insulin-dependent tissue such as liver, adipose tissue, and skeletal muscle[74]. Insulin resistance is the most important etiological factor contributing to the development of T2D[75]. There are multiple mechanisms whereby insulin resistance occurs in humans, however, systemic inflammation is one of the most recently studied. Obese subjects show a constant systemic proinflammatory state characterized by abnormally high circulating levels of TNF-α, IL-1β, IL-6, IL-12, and MCP-1[76,77]. In adipose tissue, TNF-α induces insulin resistance by activating protein-tyrosine phosphatase 1B that in turn can dephosphorylate the insulin receptor substrate-2 (IRS-2) resulting in glucose transport arrest and hyperglycemia[78,79]. IL-6 and the NF-κB pathway are also involved in insulin resistance and progressive loss of normal glucose tolerance[80,81]. In diabetic patients, NF-κB upregulates PKC-θ, AP-1, and c-Jun kinase, which all act together to inhibit the insulin receptor *via* serine/threonine phosphorylation of the IRS [82].

SARS-CoV-2 infection in pancreatic β-cells not only reduces insulin secretion but also provokes a proinflammatory cytokine storm that exacerbates insulin resistance [15]. It is well known that even mild SARS-CoV-2 infection can trigger a proinflammatory cascade that mainly increases TNF-α, IL-6, MCP-1, and IL-1β in the lungs and

blood stream[83]. Similarly, SARS-CoV-2 infection produces high levels of IFN-γ inducible protein-10 (IP-10) that can itself lower insulin sensitivity[84]. Thus, release of proinflammatory molecules in non-diabetic and diabetic patients with untreated insulin resistance might aggravate COVID-19 symptoms and increase its severity.

ACE2 is an important link between insulin resistance and severe COVID-19, since it acts as the main cellular entry point for SARS-CoV-2[85]. Under normal conditions, ACE2 converts angiotensin II into angiotensin 1-7 in order to prevent angiotensin II-related physiological disturbances such as vasoconstriction, inflammation, oxidative stress, and insulin resistance[86]. In mice fed a high-sucrose diet, ACE2 is upregulated to remove excess angiotensin II and mitigate its negative effects on insulin sensitivity and glucose transport *via* the glucose transporter protein family[87,88]. T2D patients demonstrate increased ACE2 receptor levels that in turn may help SARS-CoV-2 extend cellular binding, thus boosting viral load and severity of infection.

Likewise, IFN-γ increases in patients with severe SARS-CoV-2 infection and reduces insulin sensitivity *via* IP-10[89]. Also, IFN-γ produced in response to multiple viral infections can cause insulin resistance in skeletal muscle and adipose tissue by downregulating PI3K[90,91]. It is thus reasonable to speculate that increased IFN-gamma production in patients with COVID-19 may aggravate pre-existing insulin resistance in both non-diabetic and diabetic patients.

Insulin resistance also seems to prevent the anti-inflammatory T-helper type 2 differentiation of CD4+ lymphocytes *via* the extracellular signal-regulated kinase[92]. In fact, CD4+ T cells appear to induce abnormal responses to insulin in conditions characterized by insulin resistance such as obesity and T2D[93]. Insulin resistance can also influence macrophages, an immune cell type thought to play a key role in preventing COVID-19-related organ damage[94]. Consistent with these findings, monocytes and macrophages that lack insulin signaling show impaired responses to a variety of pathogens[95]. In T2D patients, insulin resistance is also associated with high blood neutrophil count, which is of particular importance in severe COVID-19 that is characterized by neutrophilia and monocytopenia[18,96].

FORMATION OF ADVANCED GLYCATION END PRODUCTS

Pathogenesis of T2D is also characterized by the non-enzymatic covalent attachment of glucose to molecules such as proteins, lipids, and/or nucleic acids, a process that results in the formation of AGEs[97]. In addition to their negative effects on the insulin signaling pathway, AGEs have been shown to bind several surface receptors such as CD36, scavenger receptors type I and II, and galectin-3[98,99]. Upon receptor recognition, AGEs stimulate the release of pro-inflammatory cytokines in lymphocytes, monocytes, and macrophages and promote vascular inflammation and endothelial dysfunction[101]. AGEs can also directly bind to the receptor for advanced glycation end products (RAGE), a multi-ligand binding protein that promotes sustained inflammatory responses[100]. Notably, the lungs can express high RAGE levels, which may increase pulmonary inflammation in T2D patients in whom a wide variety of AGEs are produced[101]. RAGE is expressed in alveolar epithelial cells, vascular smooth muscle pulmonary cells, airway smooth muscle cells, and endothelial cells[102-105]. The AGE-RAGE interaction activates the NLRP3 inflammasome pathway. NLRP3 polarizes macrophages toward M1, inducing neutrophil extracellular trap formation and increasing the Th17 Lymphocyte population. Altogether, these inflammatory actions can perpetuate the cytokine storm, leading to pulmonary inflammation and fibrosis in patients with COVID-19[101,106]. This hypothesis is supported by the finding that RAGE-dependent inflammatory pathways play a detrimental role in pneumonia and ARDS[107].

AGEs produced in diabetic patients are also known to activate the classical complement pathway by recognizing C1q, which in turn inactivates CD59 and increases vascular injury in blood vessels of T2D patients[108]. In agreement with this concept, membrane attack complex deposits present in lung tissue from patients with severe COVID-19 has revealed complement-mediated damage which in turn induces vascular inflammation and results in extended lung damage[109].

It is well known that excess glucose can be non-enzymatically attached to hemoglobin to form HbA1c. This is particularly relevant to T2D and COVID-19 since SARS-CoV-2 is capable of altering the 1-beta chain of hemoglobin. This causes iron dissociation and porphyrin formation, thus affecting oxygen affinity and bioavailability in peripheral tissues[110]. It follows that excess glycation of hemoglobin in T2D patients may contribute to breathing difficulty that progresses to ARDS, a key

pathophysiological component of severe COVID-19. Indeed, a recent study reported that ACE2 can be glycated in hyperglycemic conditions[111]. Interestingly, ACE2 glycation appears to increase SARS-CoV-2 affinity and entry into pancreatic and lung tissue[112,113]. Good glycemic control has been shown to lower the amount of glycated ACE2 in lung tissue, ameliorating pneumonia and COVID-19 severity presumably by reducing the availability of viral entry points[113]. Conversely, uncontrolled hyperglycemia leads to aberrant formation of glycated ACE2 not only in lungs but also in nasal airways, tongue, and oropharynx, which may increase viral entry points and disease severity[113].

Last but not least, CD147 is a glycoprotein expressed in type II pneumocytes that binds the spike S1 protein, thus favoring SARS-CoV-2 entry into lung cells[114]. Evidence in T2D patients suggests that CD147 can be glycated in hyperglycemic conditions, which is linked to metalloproteinase upregulation and loss of tight junctions that may favor cell entry of SARS-CoV-2 and increase viral load[115]. As we have outlined, formation of AGEs appears to play a key role in the severity of COVID-19, which becomes more relevant in T2D patients with poor glycemic control, a condition that favors protein glycation.

ENDOTHELIAL DYSFUNCTION, VASCULAR DAMAGE, AND PROTHROMBOTIC STATE

The vascular endothelium maintains homeostasis by modulating blood flow, fybrinolysis, coagulation, platelet adherence, and immune cell trafficking in response to cell injury[116]. Impaired vascular endothelium function in patients with diabetes mellitus is considered a risk factor for cardiovascular disease[117]. Emerging evidence suggests that COVID-19 aggravates vascular pathology due to proliferation of SARS-CoV-2 in endothelial cells. This induces cellular damage, apoptosis, and disruption of the vascular barrier, which is especially relevant in T2D patients that show impaired angiogenesis[118-120]. Notably, endothelial dysfunction is a central feature in COVID-19 pathogenesis[121]. Patients with COVID-19 have nitric oxide (NO) deficiencies that lead to increased vascular contraction and reduced ROS neutralization[122,123]. Upon SARS-CoV-2 infection, the vascular endothelium undergoes vascular leakage and enhanced blood clotting. Subsequent recruitment of immune mediators results in inflammation that perpetuates tissue damage and vascular impairment[124]. As mentioned above, COVID-19 is accompanied by a high number of neutrophils, proinflammatory immune cells that also contribute to vascular damage in T2D. Diabetic patients have neutrophils with enhanced oxidative activity that produce high free radical levels and neutrophil extracellular traps (NETs) which can cause direct injury to blood vessels[125,126]. Neutrophils are also major producers of myeloperoxidase, a peroxidase enzyme that binds to the vascular endothelium and increases blood vessel damage in T2D patients[127]. These lines of evidence support a deleterious synergistic effect of T2D on COVID-19, wherein hyper-reactive neutrophils may directly injure the vascular endothelium and worsen the patient's outcome[96]. Interestingly, SARS-CoV-2 can increase NET release by infecting neutrophils. Increased formation of NETs can then directly injure the lung epithelium.

Proinflammatory cytokines play a decisive role in endothelial dysfunction. It is well known that IL-6 is upregulated in T2D and is associated with endothelial damage and atherosclerosis[128]. In patients with severe COVID-19, IL-6 induces chemokine expression, leukocyte trafficking, immune cell extravasation toward arterial walls, NO reduction, increased oxidative stress, and exacerbated inflammation of blood vessels [129]. TNF-α, another important proinflammatory cytokine involved in the COVID-19 cytokine storm, accelerates atherosclerosis *via* vascular cell adhesion molecule-1, E-selectin, and MCP1, which impairs vasodilatation and promotes endothelial cell apoptosis[81]. Post-mortem examination of lung tissue from patients severely infected with SARS-CoV-2 revealed massive mononuclear and polymorphonuclear cell infiltration, supporting the role of immune cell recruitment in COVID-19 progression[118]. These findings indicate that chemoattraction and recruitment of immune cells act together with endothelial dysfunction to induce vascular damage in patients with T2D, which may worsen the severity of COVID-19 by increasing vascular leaking and prothrombosis.

Disruption of vascular integrity promotes basement membrane exposure, coagulopathy, D-dimer release, and fibrinogen and platelet activation, all of which are important biomarkers for poor prognosis in COVID-19[130-133]. It is well known that the prothrombotic state common in patients with T2D may increase the occurrence of

severe coagulopathy in patients critically ill with COVID-19[134]. In fact, the severity of COVID-19 increases in parallel with pulmonary embolism, microcirculatory malfunction, and disseminated intravascular coagulation[135,136]. Microvascular and macrovascular thromboembolic events have been documented in the kidneys, lungs, spleen, and brains of SARS-CoV-2 infected patients[137-140]. Thrombotic incidence of about 30% has been reported in lungs[141] and incidence of deep venous thrombosis as occurs in the lower limbs of T2D patients has been reported at 46%[142].

The mechanisms underlying thromboembolic events in T2D patients with severe COVID-19 remain unclear, but persistent inflammation has now emerged as a potential contributor[143]. As described above, levels of several proinflammatory cytokines, including TNF-α, IL-6, and IL-8 are elevated in patients with COVID-19 who required hospitalization[144,145]. Interestingly, some of these cytokines have prothrombotic effects by themselves[146]. For instance, there is a positive association between elevated IL-6 and increased fibrinogen levels[146]. During sepsis, monocytes and macrophages release TNF-α as well as tissue factors that activate clotting pathways[147]. Besides inducing proinflammatory cytokine production, SARS-CoV2 has been shown to induce expression of procoagulant genes such as fibrinogen, tissue factor, factor II, and factor X[148,149] *in vitro* culture models. During numerous viral infections and sepsis, activation of the innate immune system leads to increased activation of the complement system, von Willebrand factor, tissue factor, and factor VIIa[148,150,151]. Likewise, complement activation during COVID-19-related sepsis intensifies the cytokine storm and perpetuates microvascular damage[152].

T2D increases mortality risk in COVID-19 due to the preexisting prothrombotic state secondary to diabetes, where hyperglycemia by itself appears to play a contributing role[151]. Human aortal endothelial cells cultured in high glucose *in vitro* were shown to trigger both inflammatory and prothrombotic pathways[153]. Similarly, hyperglycemia acts in synergy with neutrophils to release calprotectin, a protein that can bind RAGE on Kupffer cells and induce IL-6 synthesis. IL-6 increases thrombopoietin production, which enhances proliferation and expansion of thrombotic precursors and leads to thrombocytosis[154]. Similarly, P2Y12, a receptor expressed on the surface of platelets that plays essential roles in platelet activation, may be elevated in T2D patients, and facilitate platelet adhesion to vascular endothelium[155,156]. Consistent with these findings, numerous reports have demonstrated that the prothrombotic state can be mitigated by lowering blood glucose concentration[154]. Thus, several factors associated with T2D including endothelial dysfunction, vascular damage, systemic inflammation, and hyperglycemia can directly aggravate the prothrombotic state and increase mortality risk in patients with severe COVID-19[138,141,157].

CONCLUSION

COVID-19 is an ongoing global pandemic that has challenged the ability of healthcare providers to treat the most vulnerable patient populations, such as those living with preexisting T2D. Indeed, managing the syndemic between the two current pandemics of COVID-19 and T2D has become a major contemporary public health challenge. We have shown that T2D acts in synergy with SARS-CoV-2 infection to accelerate disease progression, increase severity, and heighten the mortality risk of COVID-19. We have discussed the mechanisms whereby hyperglycemia contributes to the "cytokine storm" characteristic of severe SARS-CoV-2 infection by stimulating monocytes and macrophages to produce IL-1β, IL-6, and TNF-α in the airway epithelium. The main mechanisms whereby hyperglycemia promotes ROS release, inhibition of T cell activation, and NET formation in the lungs of patients with severe SARS-CoV-2 infection have also been examined. We have reviewed the molecular mechanisms by which proinflammatory cytokines induce insulin resistance and exert deleterious effects on pancreatic β-cell exhaustion in T2D patients critically ill with COVID-19. We have also studied the effect of excess glucose on AGE formation and the role of AGEs in perpetuating pneumonia and ARDS. Finally, we have discussed the contribution of preexisting endothelial dysfunction secondary to diabetes to the development of neutrophil trafficking, vascular leaking, and thrombotic events in patients with severe SARS-CoV-2 infection (Table 1). We have not, however, addressed the possible contribution of other components of T2D such as nerve injury, hyperglucagonemia, adiposity, dyslipidemia, endoplasmic reticulum stress, glomerular and myocardial damage, and hypovitaminosis D to the severity of COVID-19. Importantly, the efficacy of vaccines against SARS-CoV-2 should be rigorously scrutinized in patients with T2D,

Table 1 Immunometabolic mechanisms of the main etiological factors associated with type 2 diabetes and their implications in the development of severe severe acute respiratory syndrome coronavirus-2 infection

Etiological component of T2D	Effect on immune responses	Implications in COVID-19	Ref.
Hyperglycemia	Stimulation of monocytes and macrophages to release IL-1β, IL-6, and TNF-α	Promotion of the cytokine storm and exacerbated inflammatory responses	Nielsen *et al*[23], Blair *et al*[24]
	Mitochondrial oxidative stress and production of reactive oxygen species	Activation of the proinflammatory cytokine storm	Robertson *et al*[39]
	Stimulation of lactate dehydrogenase activity	Upregulation of lactate pathway during severe COVID-19	Zhang *et al*[40]
	Increased NK cells with low levels of NKG2D and NKp46	Decreased degranulation and inefficient antiviral activity	Berrou *et al*[47]
	Low number of dendritic cells	Inefficient antigen presentation and decreased T cell activation	Zhong *et al*[52]
	Inhibition of T cell activation and proliferation	Increased viral load and COVID-19 progression	Macia et *al*[51]
	Decreased neutrophil migration and phagocytosis	Impaired viral clearance	Alba-Loureiro *et al*[53]
	Low number of IFN-γ-producing cells	Impaired antiviral response	Kalantar *et al*[54]
	Reduction of antibody titers	Inability to kill infected cells and increased viral load	Mathews *et al*[55]
Pancreatic β-cell exhaustion and hyperinsulinemia	β-cell apoptosis	Enhanced pancreatic damage through SARS-CoV-2 direct binding to ACE2 in β-cells	Weir[57]
	β-cell dysfunction through endoplasmic reticulum stress	Increased pancreatic inflammation	Butler *et al*[56]
	M1-like macrophage infiltration	Islet fibrosis and β-cell mass loss	Inoue *et al*[58], Westwell-Roper *et al*[59]
	Impaired insulin production	Increased hyperglycemia and promotion of proinflammatory cell activation	Zheng *et al*[64]
	Deterioration of exocrine pancreas	Increased pancreatic inflammation	Hayden *et al*[66]
Insulin resistance	Stimulation of proinflammatory cytokine release into circulation	Exacerbated systemic inflammation	Tabák *et al*[75], Akbari *et al*[80]
	Inactivation of the insulin signaling pathway *via* NF-κB	Suppression of IP-10 production and reduced insulin sensitivity	Antuna-Puente *et al*[81]
	Increased ACE2 receptor levels	Increased viral load and COVID-19 progression	Kuba *et al*[85]
	Decreased Th2 cell differentiation	Reduction of lymphocytes with anti-inflammatory functions	Viardot *et al*[92]
	Impaired ability of macrophages to respond to pathogens	Monocytopenia, COVID-19 progression, increased mortality risk	Rizo-Téllez *et al*[96]
	High blood neutrophil count	Neutrophilia, COVID-19 progression, increased mortality risk	DeFronzo *et al*[16]
Advanced glycation end products	Activation of the RAGE and sustained inflammatory responses	Increased pulmonary inflammation and mortality risk	Oczypok *et al*[101]
	Increased Th17 lymphocytes	Perpetuation of the cytokine storm and pulmonary inflammation	Wang *et al*[30]
	Activation of the classical complement pathway	Complement-mediated damage and membrane attack complex formation in lung tissue	Lupu *et al*[150]
	Non-enzymatic attachment of glucose to hemoglobin	Alteration of the hemoglobin 1-β chain, less oxygen bioavailability in peripheral tissues and breathing difficulty	Means[110]
	Non-enzymatic attachment of glucose to ACE2	Increased SARS-CoV-2 affinity and infection in pancreatic and lung tissue	Zhao *et al*[112], Bao *et al*[114]
	Glycation of CD147 in type II	Promotion of SARS-CoV-2 cell entry and	De Francesco *et al*[115]

	pneumocytes	increased viral load in pneumocytes	
	Neutrophil trafficking impairment	Hyper-reactive neutrophils that injure the vascular endothelium	Kraakman *et al*[154]
Endothelial dysfunction and prothrombotic state	Increased prothrombotic state	Enhanced blood clotting and severe coagulopathy	McFadyen *et al*[134]
	Hyper-activation of neutrophils in blood vessels	Vascular damage, blood vessel leaking, and sepsis	Joshi *et al*[126]
	Impaired vasodilatation with release of IL-6 and TNF-α	Microcirculatory malfunction and increased fibrinogen levels	Chi *et al*[29], Mangalmurti *et al*[27]
	Recruitment of immune cells	Blood vessel leaking and thrombosis	Ranucci *et al*[146]
	IL-6 production	Increased thrombopoietin production	Kraakman *et al*[154]
	Increased P2Y12 platelet receptor	Enhanced platelet adhesion and thrombosis	Dorsam *et al*[155]

Summary of the main immunometabolic mechanisms by which immune cells and cytokines act in synergy with preexisting hyperglycemia, β-cell dysfunction, hyperinsulinemia, insulin resistance, advanced glycation end products, endothelial dysfunction, and prothrombotic state to increase the severity, progression, and mortality of coronavirus disease 2019 in patients with type 2 diabetes. COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; T2D: Type 2 diabetes; NK: Natural killer; ACE2: Angiotensin-converting enzyme 2; IP-10: IFN-γ inducible protein-10; RAGE: Receptor for advanced glycation end products.

with careful consideration for all of the factors discussed herein. A better understanding of the T2D-related immunometabolic agents that contribute to exacerbate the severity of COVID-19 will improve our ability to identify patients with high mortality risk and prevent adverse outcomes.

REFERENCES

1 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]

2 **Ceccarelli M**, Berretta M, Venanzi Rullo E, Nunnari G, Cacopardo B. Differences and similarities between Severe Acute Respiratory Syndrome (SARS)-CoronaVirus (CoV) and SARS-CoV-2. Would a rose by another name smell as sweet? *Eur Rev Med Pharmacol Sci* 2020; **24**: 2781-2783 [PMID: 32196628 DOI: 10.26355/eurrev_202003_20551]

3 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

4 **Centers for Disease Control and Prevention**. Symptomps of Coronavirus. COVID-19 Testing Overview [cited 22 Dec 2020]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html#:~:text=Symptoms%20may%20appear%202%2D14,Cough

5 **Adhikari SP**, Meng S, Wu YJ, Mao YP, Ye RX, Wang QZ, Sun C, Sylvia S, Rozelle S, Raat H, Zhou H. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. *Infect Dis Poverty* 2020; **9**: 29 [PMID: 32183901 DOI: 10.1186/s40249-020-00646-x]

6 **Zou L**, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med* 2020; **382**: 1177-1179 [PMID: 32074444 DOI: 10.1056/NEJMc2001737]

7 **Liu Y**, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LLM, Zhang W. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis* 2020; **20**: 656-657 [PMID: 32199493 DOI: 10.1016/S1473-3099(20)30232-2]

8 **Lai CC**, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J Antimicrob Agents* 2020; **55**: 105924 [PMID: 32081636 DOI: 10.1016/j.ijantimicag.2020.105924]

9 **Khan M**, Khan H, Khan S, Nawaz M. Epidemiological and clinical characteristics of coronavirus disease (COVID-19) cases at a screening clinic during the early outbreak period: a single-centre study. *J Med Microbiol* 2020; **69**: 1114-1123 [PMID: 32783802 DOI: 10.1099/jmm.0.001231]

10 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult

inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]

11 **Yang JK**, Feng Y, Yuan MY, Yuan SY, Fu HJ, Wu BY, Sun GZ, Yang GR, Zhang XL, Wang L, Xu X, Xu XP, Chan JC. Plasma glucose levels and diabetes are independent predictors for mortality and morbidity in patients with SARS. *Diabet Med* 2006; **23**: 623-628 [PMID: 16759303 DOI: 10.1111/j.1464-5491.2006.01861.x]

12 **Li B**, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol* 2020; **109**: 531-538 [PMID: 32161990 DOI: 10.1007/s00392-020-01626-9]

13 **Knapp S**. Diabetes and infection: is there a link? *Gerontology* 2013; **59**: 99-104 [PMID: 23182884 DOI: 10.1159/000345107]

14 **Wu Z**, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020; **323**: 1239-1242 [PMID: 32091533 DOI: 10.1001/jama.2020.2648]

15 **Onder G**, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *JAMA* 2020; **323**: 1775-1776 [PMID: 32203977 DOI: 10.1001/jama.2020.4683]

16 **DeFronzo RA**, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI, Simonson DC, Testa MA, Weiss R. Type 2 diabetes mellitus. *Nat Rev Dis Primers* 2015; **1**: 15019 [PMID: 27189025 DOI: 10.1038/nrdp.2015.19]

17 **Daryabor G**, Kabelitz D, Kalantar K. An update on immune dysregulation in obesity-related insulin resistance. *Scand J Immunol* 2019; **89**: e12747 [PMID: 30593678 DOI: 10.1111/sji.12747]

18 **Feng YM**, Zhao D, Zhang N, Yu CG, Zhang Q, Thijs L, Staessen JA. Insulin Resistance in Relation to Lipids and Inflammation in Type-2 Diabetic Patients and Non-Diabetic People. *PLoS One* 2016; **11**: e0153171 [PMID: 27073920 DOI: 10.1371/journal.pone.0153171]

19 **Sattar N**, Gill JM. Type 2 diabetes as a disease of ectopic fat? *BMC Med* 2014; **12**: 123 [PMID: 25159817 DOI: 10.1186/s12916-014-0123-4]

20 **Yagihashi S**, Mizukami H, Sugimoto K. Mechanism of diabetic neuropathy: Where are we now and where to go? *J Diabetes Investig* 2011; **2**: 18-32 [PMID: 24843457 DOI: 10.1111/j.2040-1124.2010.00070.x]

21 **Di Fatta C**, Spiga R, Mancuso E, Fuoco A, Averta C, Mannino GC, Andreozzi F, Sesti G. Glucagon induces the expression of inflammatory markers in the liver through NLRP3 inflammasome. Session: Inflammatory events in type 2 diabetes. 2018 Oct 2; Berlin, Germany

22 **Leite NC**, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009; **29**: 113-119 [PMID: 18384521 DOI: 10.1111/j.1478-3231.2008.01718.x]

23 **Nielsen TB**, Pantapalangkoor P, Yan J, Luna BM, Dekitani K, Bruhn K, Tan B, Junus J, Bonomo RA, Schmidt AM, Everson M, Duncanson F, Doherty TM, Lin L, Spellberg B. Diabetes Exacerbates Infection *via* Hyperinflammation by Signaling through TLR4 and RAGE. *mBio* 2017; **8** [PMID: 28830942 DOI: 10.1128/mBio.00818-17]

24 **Blair M**. Diabetes Mellitus Review. *Urol Nurs* 2016; **36**: 27-36 [PMID: 27093761]

25 **Muniyappa R**, Gubbi S. COVID-19 pandemic, coronaviruses, and diabetes mellitus. *Am J Physiol Endocrinol Metab* 2020; **318**: E736-E741 [PMID: 32228322 DOI: 10.1152/ajpendo.00124.2020]

26 **Ye Q**, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect* 2020; **80**: 607-613 [PMID: 32283152 DOI: 10.1016/j.jinf.2020.03.037]

27 **Mangalmurti N**, Hunter CA. Cytokine Storms: Understanding COVID-19. *Immunity* 2020; **53**: 19-25 [PMID: 32610079 DOI: 10.1016/j.immuni.2020.06.017]

28 **Hu B**, Huang S, Yin L. The cytokine storm and COVID-19. *J Med Virol* 2021; **93**: 250-256 [PMID: 32592501 DOI: 10.1002/jmv.26232]

29 **Chi Y**, Ge Y, Wu B, Zhang W, Wu T, Wen T, Liu J, Guo X, Huang C, Jiao Y, Zhu F, Zhu B, Cui L. Serum Cytokine and Chemokine Profile in Relation to the Severity of Coronavirus Disease 2019 in China. *J Infect Dis* 2020; **222**: 746-754 [PMID: 32563194 DOI: 10.1093/infdis/jiaa363]

30 **Wang J**, Jiang M, Chen X, Montaner LJ. Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. *J Leukoc Biol* 2020; **108**: 17-41 [PMID: 32534467 DOI: 10.1002/JLB.3COVR0520-272R]

31 **Yang X**, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; **8**: 475-481 [PMID: 32105632 DOI: 10.1016/S2213-2600(20)30079-5]

32 **Zhang C**, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. *Int J Antimicrob Agents* 2020; **55**: 105954 [PMID: 32234467 DOI: 10.1016/j.ijantimicag.2020.105954]

33 **Bode B**, Garrett V, Messler J, McFarland R, Crowe J, Booth R, Klonoff DC. Glycemic Characteristics and Clinical Outcomes of COVID-19 Patients Hospitalized in the United States. *J Diabetes Sci Technol* 2020; **14**: 813-821 [PMID: 32389027 DOI: 10.1177/1932296820924469]

34 **Ceriello A**, Zarich SW, Testa R. Lowering glucose to prevent adverse cardiovascular outcomes in a critical care setting. *J Am Coll Cardiol* 2009; **53**: S9-13 [PMID: 19179217 DOI:

10.1016/j.jacc.2008.09.054]
35 **Ceriello A**. Acute hyperglycaemia: a 'new' risk factor during myocardial infarction. *Eur Heart J* 2005; **26**: 328-331 [PMID: 15618047 DOI: 10.1093/eurheartj/ehi049]
36 **Singh AK**, Singh R. Hyperglycemia without diabetes and new-onset diabetes are both associated with poorer outcomes in COVID-19. *Diabetes Res Clin Pract* 2020; **167**: 108382 [PMID: 32853686 DOI: 10.1016/j.diabres.2020.108382]
37 **Liao WI**, Wang JC, Chang WC, Hsu CW, Chu CM, Tsai SH. Usefulness of Glycemic Gap to Predict ICU Mortality in Critically Ill Patients With Diabetes. *Medicine (Baltimore)* 2015; **94**: e1525 [PMID: 26356728 DOI: 10.1097/MD.0000000000001525]
38 **Iacobellis G**, Penaherrera CA, Bermudez LE, Bernal Mizrachi E. Admission hyperglycemia and radiological findings of SARS-CoV2 in patients with and without diabetes. *Diabetes Res Clin Pract* 2020; **164**: 108185 [PMID: 32360710 DOI: 10.1016/j.diabres.2020.108185]
39 **Robertson RP**, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* 2003; **52**: 581-587 [PMID: 12606496 DOI: 10.2337/diabetes.52.3.581]
40 **Zhang ZY**, Miao LF, Qian LL, Wang N, Qi MM, Zhang YM, Dang SP, Wu Y, Wang RX. Molecular Mechanisms of Glucose Fluctuations on Diabetic Complications. *Front Endocrinol (Lausanne)* 2019; **10**: 640 [PMID: 31620092 DOI: 10.3389/fendo.2019.00640]
41 **Lowell BB**, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. *Science* 2005; **307**: 384-387 [PMID: 15662004 DOI: 10.1126/science.1104343]
42 **Codo AC**, Davanzo GG, Monteiro LB, de Souza GF, Muraro SP, Virgilio-da-Silva JV, Prodonoff JS, Carregari VC, de Biagi Junior CAO, Crunfli F, Jimenez Restrepo JL, Vendramini PH, Reis-de-Oliveira G, Bispo Dos Santos K, Toledo-Teixeira DA, Parise PL, Martini MC, Marques RE, Carmo HR, Borin A, Coimbra LD, Boldrini VO, Brunetti NS, Vieira AS, Mansour E, Ulaf RG, Bernardes AF, Nunes TA, Ribeiro LC, Palma AC, Agrela MV, Moretti ML, Sposito AC, Pereira FB, Velloso LA, Vinolo MAR, Damasio A, Proença-Módena JL, Carvalho RF, Mori MA, Martins-de-Souza D, Nakaya HI, Farias AS, Moraes-Vieira PM. Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a HIF-1α/Glycolysis-Dependent Axis. *Cell Metab* 2020; **32**: 437-446. e5 [PMID: 32697943 DOI: 10.1016/j.cmet.2020.07.007]
43 **Ceriello A**. Hyperglycemia and COVID-19: What was known and what is really new? *Diabetes Res Clin Pract* 2020; **167**: 108383 [PMID: 32853690 DOI: 10.1016/j.diabres.2020.108383]
44 **Zhang W**, Wang G, Xu ZG, Tu H, Hu F, Dai J, Chang Y, Chen Y, Lu Y, Zeng H, Cai Z, Han F, Xu C, Jin G, Sun L, Pan BS, Lai SW, Hsu CC, Xu J, Chen ZZ, Li HY, Seth P, Hu J, Zhang X, Li H, Lin HK. Lactate Is a Natural Suppressor of RLR Signaling by Targeting MAVS. *Cell* 2019; **178**: 176-189. e15 [PMID: 31155231 DOI: 10.1016/j.cell.2019.05.003]
45 **Li X**, Xu S, Yu M, Wang K, Tao Y, Zhou Y, Shi J, Zhou M, Wu B, Yang Z, Zhang C, Yue J, Zhang Z, Renz H, Liu X, Xie J, Xie M, Zhao J. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020; **146**: 110-118 [PMID: 32294485 DOI: 10.1016/j.jaci.2020.04.006]
46 **Piątkiewicz P**, Miłek T, Bernat-Karpińska M, Ohams M, Czech A, Ciostek P. The dysfunction of NK cells in patients with type 2 diabetes and colon cancer. *Arch Immunol Ther Exp (Warsz)* 2013; **61**: 245-253 [PMID: 23456207 DOI: 10.1007/s00005-013-0222-5]
47 **Berrou J**, Fougeray S, Venot M, Chardiny V, Gautier JF, Dulphy N, Toubert A, Peraldi MN. Natural killer cell function, an important target for infection and tumor protection, is impaired in type 2 diabetes. *PLoS One* 2013; **8**: e62418 [PMID: 23638076 DOI: 10.1371/journal.pone.0062418]
48 **Angelidi AM**, Belanger MJ, Mantzoros CS. Commentary: COVID-19 and diabetes mellitus: What we know, how our patients should be treated now, and what should happen next. *Metabolism* 2020; **107**: 154245 [PMID: 32320742 DOI: 10.1016/j.metabol.2020.154245]
49 **Summers KL**, Marleau AM, Mahon JL, McManus R, Hramiak I, Singh B. Reduced IFN-alpha secretion by blood dendritic cells in human diabetes. *Clin Immunol* 2006; **121**: 81-89 [PMID: 16859998 DOI: 10.1016/j.clim.2006.05.015]
50 **Gilardini Montani MS**, Granato M, Cuomo L, Valia S, Di Renzo L, D'Orazi G, Faggioni A, Cirone M. High glucose and hyperglycemic sera from type 2 diabetic patients impair DC differentiation by inducing ROS and activating Wnt/β-catenin and p38 MAPK. *Biochim Biophys Acta* 2016; **1862**: 805-813 [PMID: 26769359 DOI: 10.1016/j.bbadis.2016.01.001]
51 **Macia L**, Delacre M, Abboud G, Ouk TS, Delanoye A, Verwaerde C, Saule P, Wolowczuk I. Impairment of dendritic cell functionality and steady-state number in obese mice. *J Immunol* 2006; **177**: 5997-6006 [PMID: 17056524 DOI: 10.4049/jimmunol.177.9.5997]
52 **Zhong J**, Tang J, Ye C, Dong L. The immunology of COVID-19: is immune modulation an option for treatment? *Lancet Rheumatol* 2020; **2**: e428-e436 [PMID: 32835246 DOI: 10.1016/S2665-9913(20)30120-X]
53 **Alba-Loureiro TC**, Munhoz CD, Martins JO, Cerchiaro GA, Scavone C, Curi R, Sannomiya P. Neutrophil function and metabolism in individuals with diabetes mellitus. *Braz J Med Biol Res* 2007; **40**: 1037-1044 [PMID: 17665039 DOI: 10.1590/S0100-879X2006005000143]
54 **Kalantar F**, Dabbaghmanesh MH, Martinuzzi E, Moghadami M, Amirghofran Z. Islet amyloid polypeptide is not a target antigen for CD8+ T-cells in type 2 diabetes. *Iran J Immunol* 2014; **11**: 1-12 [PMID: 24632583]
55 **Mathews CE**, Brown EL, Martinez PJ, Bagaria U, Nahm MH, Burton RL, Fisher-Hoch SP, McCormick JB, Mirza S. Impaired function of antibodies to pneumococcal surface protein A but not

to capsular polysaccharide in Mexican American adults with type 2 diabetes mellitus. *Clin Vaccine Immunol* 2012; **19**: 1360-1369 [PMID: 22761295 DOI: 10.1128/CVI.00268-12]

56 **Butler AE**, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003; **52**: 102-110 [PMID: 12502499 DOI: 10.2337/diabetes.52.1.102]

57 **Weir GC**. Glucolipotoxicity, β-Cells, and Diabetes: The Emperor Has No Clothes. *Diabetes* 2020; **69**: 273-278 [PMID: 31519699 DOI: 10.2337/db19-0138]

58 **Inoue H**, Shirakawa J, Togashi Y, Tajima K, Okuyama T, Kyohara M, Tanaka Y, Orime K, Saisho Y, Yamada T, Shibue K, Kulkarni RN, Terauchi Y. Signaling between pancreatic β cells and macrophages *via* S100 calcium-binding protein A8 exacerbates β-cell apoptosis and islet inflammation. *J Biol Chem* 2018; **293**: 5934-5946 [PMID: 29496993 DOI: 10.1074/jbc.M117.809228]

59 **Westwell-Roper CY**, Ehses JA, Verchere CB. Resident macrophages mediate islet amyloid polypeptide-induced islet IL-1β production and β-cell dysfunction. *Diabetes* 2014; **63**: 1698-1711 [PMID: 24222351 DOI: 10.2337/db13-0863]

60 **Guardado-Mendoza R**, Davalli AM, Chavez AO, Hubbard GB, Dick EJ, Majluf-Cruz A, Tene-Perez CE, Goldschmidt L, Hart J, Perego C, Comuzzie AG, Tejero ME, Finzi G, Placidi C, La Rosa S, Capella C, Halff G, Gastaldelli A, DeFronzo RA, Folli F. Pancreatic islet amyloidosis, beta-cell apoptosis, and alpha-cell proliferation are determinants of islet remodeling in type-2 diabetic baboons. *Proc Natl Acad Sci USA* 2009; **106**: 13992-13997 [PMID: 19666551 DOI: 10.1073/pnas.0906471106]

61 **Jallut D**, Golay A, Munger R, Frascarolo P, Schutz Y, Jéquier E, Felber JP. Impaired glucose tolerance and diabetes in obesity: a 6-year follow-up study of glucose metabolism. *Metabolism* 1990; **39**: 1068-1075 [PMID: 2215253 DOI: 10.1016/0026-0495(90)90168-C]

62 **Hayden MR**. An Immediate and Long-Term Complication of COVID-19 May Be Type 2 Diabetes Mellitus: The Central Role of β-Cell Dysfunction, Apoptosis and Exploration of Possible Mechanisms. *Cells* 2020; **9** [PMID: 33202960 DOI: 10.3390/cells9112475]

63 **Yang JK**, Lin SS, Ji XJ, Guo LM. Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. *Acta Diabetol* 2010; **47**: 193-199 [PMID: 19333547 DOI: 10.1007/s00592-009-0109-4]

64 **Zheng X**, Zheng X, Wang X, Ma Z, Gupta Sunkari V, Botusan I, Takeda T, Björklund A, Inoue M, Catrina SB, Brismar K, Poellinger L, Pereira TS. Acute hypoxia induces apoptosis of pancreatic β-cell by activation of the unfolded protein response and upregulation of CHOP. *Cell Death Dis* 2012; **3**: e322 [PMID: 22695615 DOI: 10.1038/cddis.2012.66]

65 **Maddaloni E**, Buzzetti R. Covid-19 and diabetes mellitus: unveiling the interaction of two pandemics. *Diabetes Metab Res Rev* 2020; e33213321 [PMID: 32233018 DOI: 10.1002/dmrr.3321]

66 **Hayden MR**, Sowers JR. Isletopathy in Type 2 diabetes mellitus: implications of islet RAS, islet fibrosis, islet amyloid, remodeling, and oxidative stress. *Antioxid Redox Signal* 2007; **9**: 891-910 [PMID: 17516840 DOI: 10.1089/ars.2007.1610]

67 **Pina AF**, Patarrão RS, Ribeiro RT, Penha-Gonçalves C, Raposo JF, Gardete-Correia L, Duarte R, M Boavida J, L Medina J, Henriques R, Macedo MP. Metabolic Footprint, towards Understanding Type 2 Diabetes beyond Glycemia. *J Clin Med* 2020; **9** [PMID: 32785111 DOI: 10.3390/jcm9082588]

68 **Hayden MR**, Sowers JR. Redox imbalance in diabetes. *Antioxid Redox Signal* 2007; **9**: 865-867 [PMID: 17477794 DOI: 10.1089/ars.2007.1640]

69 **Hayden MR**. Endothelial activation and dysfunction in metabolic syndrome, type 2 diabetes and coronavirus disease 2019. *J Int Med Res* 2020; **48**: 300060520939746 [PMID: 32722979 DOI: 10.1177/0300060520939746]

70 **Hayden MR**, Sowers JR. Pancreatic renin-angiotensin-aldosterone system in the cardiometabolic syndrome and type 2 diabetes mellitus. *J Cardiometab Syndr* 2008; **3**: 129-131 [PMID: 18983326 DOI: 10.1111/j.1559-4572.2008.00006.x]

71 **Hayden MR**, Karuparthi PR, Habibi J, Wasekar C, Lastra G, Manrique C, Stas S, Sowers JR. Ultrastructural islet study of early fibrosis in the Ren2 rat model of hypertension. Emerging role of the islet pancreatic pericyte-stellate cell. *JOP* 2007; **8**: 725-738 [PMID: 17993725]

72 **Aloysius MM**, Thatti A, Gupta A, Sharma N, Bansal P, Goyal H. COVID-19 presenting as acute pancreatitis. *Pancreatology* 2020; **20**: 1026-1027 [PMID: 32444169 DOI: 10.1016/j.pan.2020.05.003]

73 **Ceriello A**, De Nigris V, Prattichizzo F. Why is hyperglycaemia worsening COVID-19 and its prognosis? *Diabetes Obes Metab* 2020; **22**: 1951-1952 [PMID: 32463166 DOI: 10.1111/dom.14098]

74 **Garbossa SG**, Folli F. Vitamin D, sub-inflammation and insulin resistance. A window on a potential role for the interaction between bone and glucose metabolism. *Rev Endocr Metab Disord* 2017; **18**: 243-258 [PMID: 28409320 DOI: 10.1007/s11154-017-9423-2]

75 **Tabák AG**, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet* 2009; **373**: 2215-2221 [PMID: 19515410 DOI: 10.1016/S0140-6736(09)60619-X]

76 **Daniele G**, Guardado Mendoza R, Winnier D, Fiorentino TV, Pengou Z, Cornell J, Andreozzi F, Jenkinson C, Cersosimo E, Federici M, Tripathy D, Folli F. The inflammatory status score including IL-6, TNF-α, osteopontin, fractalkine, MCP-1 and adiponectin underlies whole-body insulin

resistance and hyperglycemia in type 2 diabetes mellitus. *Acta Diabetol* 2014; **51**: 123-131 [PMID: 24370923 DOI: 10.1007/s00592-013-0543-1]

77 **Fève B**, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol* 2009; **5**: 305-311 [PMID: 19399017 DOI: 10.1038/nrendo.2009.62]

78 **Hotamisligil GS**, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996; **271**: 665-668 [PMID: 8571133 DOI: 10.1126/science.271.5249.665]

79 **Méndez-García LA**, Trejo-Millán F, Martínez-Reyes CP, Manjarrez-Reyna AN, Esquivel-Velázquez M, Melendez-Mier G, Islas-Andrade S, Rojas-Bernabé A, Kzhyshkowska J, Escobedo G. Infliximab ameliorates tumor necrosis factor-alpha-induced insulin resistance by attenuating PTP1B activation in 3T3L1 adipocytes in vitro. *Scand J Immunol* 2018; **88**: e12716 [PMID: 30260514 DOI: 10.1111/sji.12716]

80 **Akbari M**, Hassan-Zadeh V. IL-6 signalling pathways and the development of type 2 diabetes. *Inflammopharmacology* 2018; **26**: 685-698 [PMID: 29508109 DOI: 10.1007/s10787-018-0458-0]

81 **Antuna-Puente B**, Feve B, Fellahi S, Bastard JP. Adipokines: the missing link between insulin resistance and obesity. *Diabetes Metab* 2008; **34**: 2-11 [PMID: 18093861 DOI: 10.1016/j.diabet.2007.09.004]

82 **Bastard JP**, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2006; **17**: 4-12 [PMID: 16613757]

83 **Pal R**, Bhadada SK. COVID-19 and diabetes mellitus: An unholy interaction of two pandemics. *Diabetes Metab Syndr* 2020; **14**: 513-517 [PMID: 32388331 DOI: 10.1016/j.dsx.2020.04.049]

84 **Kassir R**. Risk of COVID-19 for patients with obesity. *Obes Rev* 2020; **21**: e13034 [PMID: 32281287 DOI: 10.1111/obr.13034]

85 **Kuba K**, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; **11**: 875-879 [PMID: 16007097 DOI: 10.1038/nm1267]

86 **Underwood PC**, Adler GK. The renin angiotensin aldosterone system and insulin resistance in humans. *Curr Hypertens Rep* 2013; **15**: 59-70 [PMID: 23242734 DOI: 10.1007/s11906-012-0323-2]

87 **Takeda M**, Yamamoto K, Takemura Y, Takeshita H, Hongyo K, Kawai T, Hanasaki-Yamamoto H, Oguro R, Takami Y, Tatara Y, Takeya Y, Sugimoto K, Kamide K, Ohishi M, Rakugi H. Loss of ACE2 exaggerates high-calorie diet-induced insulin resistance by reduction of GLUT4 in mice. *Diabetes* 2013; **62**: 223-233 [PMID: 22933108 DOI: 10.2337/db12-0177]

88 **Rao S**, Lau A, So HC. Exploring Diseases/Traits and Blood Proteins Causally Related to Expression of ACE2, the Putative Receptor of SARS-CoV-2: A Mendelian Randomization Analysis Highlights Tentative Relevance of Diabetes-Related Traits. *Diabetes Care* 2020; **43**: 1416-1426 [PMID: 32430459 DOI: 10.2337/dc20-0643]

89 **Lee JS**, Park S, Jeong HW, Ahn JY, Choi SJ, Lee H, Choi B, Nam SK, Sa M, Kwon JS, Jeong SJ, Lee HK, Park SH, Choi JY, Kim SH, Jung I, Shin EC. Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19. *Sci Immunol* 2020; **5** [PMID: 32651212 DOI: 10.1126/sciimmunol.abd1554]

90 **Šestan M**, Marinović S, Kavazović I, Cekinović Đ, Wueest S, Turk Wensveen T, Brizić I, Jonjić S, Konrad D, Wensveen FM, Polić B. Virus-Induced Interferon-γ Causes Insulin Resistance in Skeletal Muscle and Derails Glycemic Control in Obesity. *Immunity* 2018; **49**: 164-177. e6 [PMID: 29958802 DOI: 10.1016/j.immuni.2018.05.005]

91 **Duffaut C**, Zakaroff-Girard A, Bourlier V, Decaunes P, Maumus M, Chiotasso P, Sengenès C, Lafontan M, Galitzky J, Bouloumié A. Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. *Arterioscler Thromb Vasc Biol* 2009; **29**: 1608-1614 [PMID: 19644053 DOI: 10.1161/ATVBAHA.109.192583]

92 **Viardot A**, Grey ST, Mackay F, Chisholm D. Potential antiinflammatory role of insulin *via* the preferential polarization of effector T cells toward a T helper 2 phenotype. *Endocrinology* 2007; **148**: 346-353 [PMID: 17008395 DOI: 10.1210/en.2006-0686]

93 **Viardot A**, Heilbronn LK, Samocha-Bonet D, Mackay F, Campbell LV, Samaras K. Obesity is associated with activated and insulin resistant immune cells. *Diabetes Metab Res Rev* 2012; **28**: 447-454 [PMID: 22492715 DOI: 10.1002/dmrr.2302]

94 **Gómez-Rial J**, Rivero-Calle I, Salas A, Martinón-Torres F. Role of Monocytes/Macrophages in Covid-19 Pathogenesis: Implications for Therapy. *Infect Drug Resist* 2020; **13**: 2485-2493 [PMID: 32801787 DOI: 10.2147/IDR.S258639]

95 **Ieronymaki E**, Daskalaki MG, Lyroni K, Tsatsanis C. Insulin Signaling and Insulin Resistance Facilitate Trained Immunity in Macrophages Through Metabolic and Epigenetic Changes. *Front Immunol* 2019; **10**: 1330 [PMID: 31244863 DOI: 10.3389/fimmu.2019.01330]

96 **Rizo-Téllez SA**, Méndez-García LA, Flores-Rebollo C, Alba-Flores F, Alcántara-Suárez R, Manjarrez-Reyna AN, Baltazar-López N, Hernández-Guzmán VA, León-Pedroza JI, Zapata-Arenas R, González-Chávez A, Hernández-Ruíz J, Carrillo-Ruíz JD, Serrano-Loyola R, Guerrero-Avendaño GML, Escobedo G. The Neutrophil-to-Monocyte Ratio and Lymphocyte-to-Neutrophil Ratio at Admission Predict In-Hospital Mortality in Mexican Patients with Severe SARS-CoV-2 Infection (Covid-19). *Microorganisms* 2020; **8** [PMID: 33050487 DOI: 10.3390/microorganisms8101560]

97 **Maessen DE**, Stehouwer CD, Schalkwijk CG. The role of methylglyoxal and the glyoxalase system in diabetes and other age-related diseases. *Clin Sci (Lond)* 2015; **128**: 839-861 [PMID: 25818485 DOI: 10.1042/CS20140683]

98 **Hegab Z**, Gibbons S, Neyses L, Mamas MA. Role of advanced glycation end products in cardiovascular disease. *World J Cardiol* 2012; **4**: 90-102 [PMID: 22558488 DOI: 10.4330/wjc.v4.i4.90]

99 **Mulrennan S**, Baltic S, Aggarwal S, Wood J, Miranda A, Frost F, Kaye J, Thompson PJ. The role of receptor for advanced glycation end products in airway inflammation in CF and CF related diabetes. *Sci Rep* 2015; **5**: 8931 [PMID: 25754382 DOI: 10.1038/srep08931]

100 **Fehrenbach H**, Kasper M, Tschernig T, Shearman MS, Schuh D, Müller M. Receptor for advanced glycation endproducts (RAGE) exhibits highly differential cellular and subcellular localisation in rat and human lung. *Cell Mol Biol (Noisy-le-grand)* 1998; **44**: 1147-1157 [PMID: 9846897 DOI: 10.1161/ATVBAHA.120.315527]

101 **Oczypok EA**, Perkins TN, Oury TD. All the "RAGE" in lung disease: The receptor for advanced glycation endproducts (RAGE) is a major mediator of pulmonary inflammatory responses. *Paediatr Respir Rev* 2017; **23**: 40-49 [PMID: 28416135 DOI: 10.1016/j.prrv.2017.03.012]

102 **Katsuoka F**, Kawakami Y, Arai T, Imuta H, Fujiwara M, Kanma H, Yamashita K. Type II alveolar epithelial cells in lung express receptor for advanced glycation end products (RAGE) gene. *Biochem Biophys Res Commun* 1997; **238**: 512-516 [PMID: 9299542 DOI: 10.1006/bbrc.1997.7263]

103 **Prasad K**. AGE-RAGE Stress in the Pathophysiology of Pulmonary Hypertension and its Treatment. *Int J Angiol* 2019; **28**: 71-79 [PMID: 31384104 DOI: 10.1055/s-0039-1687818]

104 **Nakamura K**, Sakaguchi M, Matsubara H, Akagi S, Sarashina T, Ejiri K, Akazawa K, Kondo M, Nakagawa K, Yoshida M, Miyoshi T, Ogo T, Oto T, Toyooka S, Higashimoto Y, Fukami K, Ito H. Crucial role of RAGE in inappropriate increase of smooth muscle cells from patients with pulmonary arterial hypertension. *PLoS One* 2018; **13**: e0203046 [PMID: 30180189 DOI: 10.1371/journal.pone.0203046]

105 **Polverino F**, Celli BR, Owen CA. COPD as an endothelial disorder: endothelial injury linking lesions in the lungs and other organs? *Pulm Circ* 2018; **8**: 2045894018758528 [PMID: 29468936 DOI: 10.1177/2045894018758528]

106 **Ahn M**, Anderson DE, Zhang Q, Tan CW, Lim BL, Luko K, Wen M, Chia WN, Mani S, Wang LC, Ng JHJ, Sobota RM, Dutertre CA, Ginhoux F, Shi ZL, Irving AT, Wang LF. Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. *Nat Microbiol* 2019; **4**: 789-799 [PMID: 30804542 DOI: 10.1038/s41564-019-0371-3]

107 **Boteanu RM**, Uyy E, Suica VI, Antohe F. High-mobility group box 1 enhances the inflammatory process in diabetic lung. *Arch Biochem Biophys* 2015; **583**: 55-64 [PMID: 26254814 DOI: 10.1016/j.abb.2015.07.020]

108 **Chikazawa M**, Shibata T, Hatasa Y, Hirose S, Otaki N, Nakashima F, Ito M, Machida S, Maruyama S, Uchida K. Identification of C1q as a Binding Protein for Advanced Glycation End Products. *Biochemistry* 2016; **55**: 435-446 [PMID: 26731343 DOI: 10.1021/acs.biochem.5b00777]

109 **Manzo G**. COVID-19 as an Immune Complex Hypersensitivity in Antigen Excess Conditions: Theoretical Pathogenetic Process and Suggestions for Potential Therapeutic Interventions. *Front Immunol* 2020; **11**: 566000 [PMID: 33193337 DOI: 10.3389/fimmu.2020.566000]

110 **Means C**. Letter to the Editor: Mechanisms of increased morbidity and mortality of SARS-CoV-2 infection in individuals with diabetes: what this means for an effective management strategy. *Metabolism* 2020; **108**: 154254 [PMID: 32360397 DOI: 10.1016/j.metabol.2020.154254]

111 **Guo YR**, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY, Yan Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res* 2020; **7**: 11 [PMID: 32169119 DOI: 10.1186/s40779-020-00240-0]

112 **Zhao P**, Praissman JL, Grant OC, Cai Y, Xiao T, Rosenbalm KE, Aoki K, Kellman BP, Bridger R, Barouch DH, Brindley MA, Lewis NE, Tiemeyer M, Chen B, Woods RJ, Wells L. Virus-Receptor Interactions of Glycosylated SARS-CoV-2 Spike and Human ACE2 Receptor. *bioRxiv* 2020 [PMID: 32743578 DOI: 10.1101/2020.06.25.172403]

113 **Brufsky A**. Hyperglycemia, hydroxychloroquine, and the COVID-19 pandemic. *J Med Virol* 2020; **92**: 770-775 [PMID: 32293710 DOI: 10.1002/jmv.25887]

114 **Bao W**, Min D, Twigg SM, Shackel NA, Warner FJ, Yue DK, McLennan SV. Monocyte CD147 is induced by advanced glycation end products and high glucose concentration: possible role in diabetic complications. *Am J Physiol Cell Physiol* 2010; **299**: C1212-C1219 [PMID: 20810913 DOI: 10.1152/ajpcell.00228.2010]

115 **De Francesco EM**, Vella V, Belfiore A. COVID-19 and Diabetes: The Importance of Controlling RAGE. *Front Endocrinol (Lausanne)* 2020; **11**: 526 [PMID: 32760352 DOI: 10.3389/fendo.2020.00526]

116 **Pober JS**, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol* 2007; **7**: 803-815 [PMID: 17893694 DOI: 10.1038/nri2171]

117 **Almdal T**, Scharling H, Jensen JS, Vestergaard H. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up. *Arch Intern Med* 2004; **164**: 1422-1426 [PMID: 15249351 DOI: 10.1001/archinte.164.13.1422]

118 **Varga Z**, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F, Moch H. Endothelial cell infection and endotheliitis in COVID-19.

Lancet 2020; **395**: 1417-1418 [PMID: 32325026 DOI: 10.1016/S0140-6736(20)30937-5]
119 and vascular disease. *EBioMedicine* 2020; **58**: 102966 [PMID: 32829782 DOI: 10.1016/j.ebiom.2020.102966]
120 **Kahn MB**, Yuldasheva NY, Cubbon RM, Smith J, Rashid ST, Viswambharan H, Imrie H, Abbas A, Rajwani A, Aziz A, Baliga V, Sukumar P, Gage M, Kearney MT, Wheatcroft SB. Insulin resistance impairs circulating angiogenic progenitor cell function and delays endothelial regeneration. *Diabetes* 2011; **60**: 1295-1303 [PMID: 21317296 DOI: 10.2337/db10-1080]
121 **Iba T**, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of Coronavirus Disease 2019. *Crit Care Med* 2020; **48**: 1358-1364 [PMID: 32467443 DOI: 10.1097/CCM.0000000000004458]
122 **Green SJ**. Covid-19 accelerates endothelial dysfunction and nitric oxide deficiency. *Microbes Infect* 2020; **22**: 149-150 [PMID: 32425647 DOI: 10.1016/j.micinf.2020.05.006]
123 **Akerström S**, Gunalan V, Keng CT, Tan YJ, Mirazimi A. Dual effect of nitric oxide on SARS-CoV replication: viral RNA production and palmitoylation of the S protein are affected. *Virology* 2009; **395**: 1-9 [PMID: 19800091 DOI: 10.1016/j.virol.2009.09.007]
124 **Teuwen LA**, Geldhof V, Pasut A, Carmeliet P. COVID-19: the vasculature unleashed. *Nat Rev Immunol* 2020; **20**: 389-391 [PMID: 32439870 DOI: 10.1038/s41577-020-0343-0]
125 **Llorente L**, De La Fuente H, Richaud-Patin Y, Alvarado-De La Barrera C, Diaz-Borjón A, López-Ponce A, Lerman-Garber I, Jakez-Ocampo J. Innate immune response mechanisms in non-insulin dependent diabetes mellitus patients assessed by flow cytoenzymology. *Immunol Lett* 2000; **74**: 239-244 [PMID: 11064109 DOI: 10.1016/S0165-2478(00)00255-8]
126 **Joshi MB**, Baipadithaya G, Balakrishnan A, Hegde M, Vohra M, Ahamed R, Nagri SK, Ramachandra L, Satyamoorthy K. Elevated homocysteine levels in type 2 diabetes induce constitutive neutrophil extracellular traps. *Sci Rep* 2016; **6**: 36362 [PMID: 27811985 DOI: 10.1038/srep36362]
127 **Zhang C**, Yang J, Jennings LK. Leukocyte-derived myeloperoxidase amplifies high-glucose--induced endothelial dysfunction through interaction with high-glucose--stimulated, vascular non--leukocyte-derived reactive oxygen species. *Diabetes* 2004; **53**: 2950-2959 [PMID: 15504976 DOI: 10.2337/diabetes.53.11.2950]
128 **Andreozzi F**, Laratta E, Procopio C, Hribal ML, Sciacqua A, Perticone M, Miele C, Perticone F, Sesti G. Interleukin-6 impairs the insulin signaling pathway, promoting production of nitric oxide in human umbilical vein endothelial cells. *Mol Cell Biol* 2007; **27**: 2372-2383 [PMID: 17242212 DOI: 10.1128/mcb.01340-06]
129 **Didion SP**. Cellular and Oxidative Mechanisms Associated with Interleukin-6 Signaling in the Vasculature. *Int J Mol Sci* 2017; **18** [PMID: 29186034 DOI: 10.3390/ijms18122563]
130 **Siddiqi HK**, Libby P, Ridker PM. COVID-19 - A vascular disease. *Trends Cardiovasc Med* 2021; **31**: 1-5 [PMID: 33068723 DOI: 10.1016/j.tcm.2020.10.005]
131 **Sturtzel C**. Endothelial Cells. *Adv Exp Med Biol* 2017; **1003**: 71-91 [PMID: 28667554 DOI: 10.1007/978-3-319-57613-8_4]
132 **Panigada M**, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, Pesenti A, Peyvandi F, Tripodi A. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost* 2020; **18**: 1738-1742 [PMID: 32302438 DOI: 10.1111/jth.14850]
133 **Figliozzi S**, Masci PG, Ahmadi N, Tondi L, Koutli E, Aimo A, Stamatelopoulos K, Dimopoulos MA, Caforio ALP, Georgiopoulos G. Predictors of adverse prognosis in COVID-19: A systematic review and meta-analysis. *Eur J Clin Invest* 2020; **50**: e13362 [PMID: 32726868 DOI: 10.1111/eci.13362]
134 **McFadyen JD**, Stevens H, Peter K. The Emerging Threat of (Micro)Thrombosis in COVID-19 and Its Therapeutic Implications. *Circ Res* 2020; **127**: 571-587 [PMID: 32586214 DOI: 10.1161/CIRCRESAHA.120.317447]
135 **Jabaudon M**, Blondonnet R, Roszyk L, Bouvier D, Audard J, Clairefond G, Fournier M, Marceau G, Déchelotte P, Pereira B, Sapin V, Constantin JM. Soluble Receptor for Advanced Glycation End-Products Predicts Impaired Alveolar Fluid Clearance in Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* 2015; **192**: 191-199 [PMID: 25932660 DOI: 10.1164/rccm.201501-0020OC]
136 **Tang N**, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost* 2020; **18**: 1094-1099 [PMID: 32220112 DOI: 10.1111/jth.14817]
137 **Klok FA**, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers D, Kant KM, Kaptein FHJ, van Paassen J, Stals MAM, Huisman MV, Endeman H. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: An updated analysis. *Thromb Res* 2020; **191**: 148-150 [PMID: 32381264 DOI: 10.1016/j.thromres.2020.04.041]
138 **Xu X**, Chang XN, Pan HX, Su H, Huang B, Yang M, Luo DJ, Weng MX, Ma L, Nie X. [Pathological changes of the spleen in ten patients with coronavirus disease 2019(COVID-19) by postmortem needle autopsy]. *Zhonghua Bing Li Xue Za Zhi* 2020; **49**: 576-582 [PMID: 32340089 DOI: 10.3760/cma.j.cn112151-20200401-00278]
139 **Vulliamy P**, Jacob S, Davenport RA. Acute aorto-iliac and mesenteric arterial thromboses as presenting features of COVID-19. *Br J Haematol* 2020; **189**: 1053-1054 [PMID: 32353183 DOI: 10.1111/bjh.16760]
140 **Wichmann D**, Sperhake JP, Lütgehetmann M, Steurer S, Edler C, Heinemann A, Heinrich F, Mushumba H, Kniep I, Schröder AS, Burdelski C, de Heer G, Nierhaus A, Frings D, Pfefferle S,

Becker H, Bredereke-Wiedling H, de Weerth A, Paschen HR, Sheikhzadeh-Eggers S, Stang A, Schmiedel S, Bokemeyer C, Addo MM, Aepfelbacher M, Püschel K, Kluge S. Autopsy Findings and Venous Thromboembolism in Patients With COVID-19: A Prospective Cohort Study. *Ann Intern Med* 2020; **173**: 268-277 [PMID: 32374815 DOI: 10.7326/M20-2003]

141 **Cattaneo M**, Bertinato EM, Birocchi S, Brizio C, Malavolta D, Manzoni M, Muscarella G, Orlandi M. Pulmonary Embolism or Pulmonary Thrombosis in COVID-19? *Thromb Haemost* 2020; **120**: 1230-1232 [PMID: 32349132 DOI: 10.1055/s-0040-1712097]

142 **Zhang L**, Feng X, Zhang D, Jiang C, Mei H, Wang J, Zhang C, Li H, Xia X, Kong S, Liao J, Jia H, Pang X, Song Y, Tian Y, Wang B, Wu C, Yuan H, Zhang Y, Li Y, Sun W, Zhu S, Wang S, Xie Y, Ge S, Zhang L, Hu Y, Xie M. Deep Vein Thrombosis in Hospitalized Patients With COVID-19 in Wuhan, China: Prevalence, Risk Factors, and Outcome. *Circulation* 2020; **142**: 114-128 [PMID: 32421381 DOI: 10.1161/CIRCULATIONAHA.120.046702]

143 **Karakike E**, Giamarellos-Bourboulis EJ. Macrophage Activation-Like Syndrome: A Distinct Entity Leading to Early Death in Sepsis. *Front Immunol* 2019; **10**: 55 [PMID: 30766533 DOI: 10.3389/fimmu.2019.00055]

144 **Chen G**, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020; **130**: 2620-2629 [PMID: 32217835 DOI: 10.1172/JCI137244]

145 **Henry BM**, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med* 2020; **58**: 1021-1028 [PMID: 32286245 DOI: 10.1515/cclm-2020-0369]

146 **Ranucci M**, Ballotta A, Di Dedda U, Bayshnikova E, Dei Poli M, Resta M, Falco M, Albano G, Menicanti L. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *J Thromb Haemost* 2020; **18**: 1747-1751 [PMID: 32302448 DOI: 10.1111/jth.14854]

147 **Subramaniam S**, Scharrer I. Procoagulant activity during viral infections. *Front Biosci (Landmark Ed)* 2018; **23**: 1060-1081 [PMID: 28930589 DOI: 10.2741/4633]

148 **Giannis D**, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *J Clin Virol* 2020; **127**: 104362 [PMID: 32305883 DOI: 10.1016/j.jcv.2020.104362]

149 **Ng LF**, Hibberd ML, Ooi EE, Tang KF, Neo SY, Tan J, Murthy KR, Vega VB, Chia JM, Liu ET, Ren EC. A human in vitro model system for investigating genome-wide host responses to SARS coronavirus infection. *BMC Infect Dis* 2004; **4**: 34 [PMID: 15357874 DOI: 10.1186/1471-2334-4-34]

150 **Lupu F**, Keshari RS, Lambris JD, Coggeshall KM. Crosstalk between the coagulation and complement systems in sepsis. *Thromb Res* 2014; **133** Suppl 1: S28-S31 [PMID: 24759136 DOI: 10.1016/j.thromres.2014.03.014]

151 **Gragnano F**, Sperlongano S, Golia E, Natale F, Bianchi R, Crisci M, Fimiani F, Pariggiano I, Diana V, Carbone A, Cesaro A, Concilio C, Limongelli G, Russo M, Calabrò P. The Role of von Willebrand Factor in Vascular Inflammation: From Pathogenesis to Targeted Therapy. *Mediators Inflamm* 2017; **2017**: 5620314 [PMID: 28634421 DOI: 10.1155/2017/5620314]

152 **Irshad Z**, Xue M, Ashour A, Larkin JR, Thornalley PJ, Rabbani N. Activation of the unfolded protein response in high glucose treated endothelial cells is mediated by methylglyoxal. *Sci Rep* 2019; **9**: 7889 [PMID: 31133647 DOI: 10.1038/s41598-019-44358-1]

153 **Kraakman MJ**, Lee MK, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, Basu D, Heywood S, Kammoun HL, Flynn M, Whillas A, Hanssen NM, Febbraio MA, Westein E, Fisher EA, Chin-Dusting J, Cooper ME, Berger JS, Goldberg IJ, Nagareddy PR, Murphy AJ. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. *J Clin Invest* 2017; **127**: 2133-2147 [PMID: 28504650 DOI: 10.1172/JCI92450]

154 **Guo W**, Li M, Dong Y, Zhou H, Zhang Z, Tian C, Qin R, Wang H, Shen Y, Du K, Zhao L, Fan H, Luo S, Hu D. Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes Metab Res Rev* 2020; e3319 [PMID: 32233013 DOI: 10.1002/dmrr.3319]

155 **Dorsam RT**, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. *J Clin Invest* 2004; **113**: 340-345 [PMID: 14755328 DOI: 10.1172/JCI20986]

156 **Przygodzki T**, Luzak B, Kassassir H, Mnich E, Boncler M, Siewiera K, Kosmalski M, Szymanski J, Watala C. Diabetes and Hyperglycemia Affect Platelet GPIIIa Expression. Effects on Adhesion Potential of Blood Platelets from Diabetic Patients under In Vitro Flow Conditions. *Int J Mol Sci* 2020; **21** [PMID: 32370146 DOI: 10.3390/IJMS21093222]

157 **Gąsecka A**, Borovac JA, Guerreiro RA, Giustozzi M, Parker W, Caldeira D, Chiva-Blanch G. Thrombotic Complications in Patients with COVID-19: Pathophysiological Mechanisms, Diagnosis, and Treatment. *Cardiovasc Drugs Ther* 2021; **35**: 215-229 [PMID: 33074525 DOI: 10.1007/s10557-020-07084-9]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1042-1056

DOI: 10.4239/wjd.v12.i7.1042

ISSN 1948-9358 (online)

REVIEW

Spatial epidemiology of diabetes: Methods and insights

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Author contributions: Cuadros DF, Li J, Musuka G, and Awad SF designed the research study; Cuadros DF, Li J, and Awad SF performed the literature search; Cuadros DF, and Li JJ Generated the figure maps; Cuadros DF wrote the first draft of the manuscript; Li JJ, Musuka G, and Awad SF helped writing the final version of the manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: No conflict of interest to report.

Manuscript source: Invited

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Abstract

Diabetes mellitus (DM) is a growing epidemic with global proportions. It is estimated that in 2019, 463 million adults aged 20-79 years were living with DM. The latest evidence shows that DM continues to be a significant global health challenge and is likely to continue to grow substantially in the next decades, which would have major implications for healthcare expenditures, particularly in developing countries. Hence, new conceptual and methodological approaches to tackle the epidemic are long overdue. Spatial epidemiology has been a successful approach to control infectious disease epidemics like malaria and human immunodeficiency virus. The implementation of this approach has been expanded to include the study of non-communicable diseases like cancer and cardiovascular diseases. In this review, we discussed the implementation and use of spatial epidemiology and Geographic Information Systems to the study of DM. We reviewed several spatial methods used to understand the spatial structure of the disease and identify the potential geographical drivers of the spatial distribution of DM. Finally, we discussed the use of spatial epidemiology on the design and implementation of geographically targeted prevention and treatment interventions against DM.

manuscript

Specialty type: Public, environmental and occupational health

Country/Territory of origin: United States

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 10, 2021
Peer-review started: February 10, 2021
First decision: April 20, 2021
Revised: May 7, 2021
Accepted: June 4, 2021
Article in press: June 4, 2021
Published online: July 15, 2021

P-Reviewer: Cigrovski Berkovic M, Liu ZC
S-Editor: Zhang H
L-Editor: A
P-Editor: Wang LL

Key Words: Diabetes mellitus; Spatial epidemiology; Risk factors; Spatial statistics; Disease mapping

Core Tip: With more than 400 million people having diabetes mellitus (DM), this disease emerges as one of the biggest public health challenges of our current times. However, one of the most significant public health advances in the study of DM is the demonstration that it can be prevented by the implementation of effective interventions targeting the factors that exacerbate the risk of the disease. Spatially informed tailored strategies that allocate resources in the high-risk areas where the most vulnerable populations reside would be an effective approach aimed to control and reduce the burden of the disease. Spatially explicit community-level policy interventions would offer great promise in effectively addressing the obesogenic and diabetogenic environment aimed to control the global DM epidemic.

Citation: Cuadros DF, Li J, Musuka G, Awad SF. Spatial epidemiology of diabetes: Methods and insights. *World J Diabetes* 2021; 12(7): 1042-1056
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1042.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1042

INTRODUCTION

Diabetes mellitus (DM) is an illness triggered by an incapacity of the insulin generated by the pancreas to effectively transfer glucose into cells *via* transporter recruitment, leading to uncontrolled hyperglycemia[1]. The common classifications of DM are the polygenic forms type I (T1DM) and type II (T2DM)[2]. T1DM is described by the absence of insulin production generated by the autoimmune destruction of pancreatic beta cells, whereas T2DM is an acquired disorder in which the pancreas either becomes insulin deficient or sufficient insulin is produced but the body cells cannot respond to the insulin, labeled insulin resistance[3,4]. In general, more than 90% of all DM diagnoses are T2DM. Other types of DM include gestational DM (any degree of glucose intolerance during pregnancy), monogenic DM syndromes, and drug (*e.g.*, steroid) or chemical induced DM, among others[4,5]. DM increases the risk of developing different comorbidities and other health complications including cerebrovascular accidents, hypertension, retinopathy and other ocular diseases, nephropathy, cardiovascular diseases, mental health conditions (*e.g.*, anxiety and depression), skin infections, and lower-limb compromise, among others[6].

Several genetic factors trigger the development of DM[7,8]. To date, more than 60 gene variants have been linked with the development of DM, but the actual effect size of each of these individual gene variants is largely unknown[9]. Behavioral, health-related, and environmental risk factors are also linked to increased risk of DM including obesity, physical inactivity, obesogenic environments, pollution, history of gestational DM, hypertension, and dyslipidemia, among others[10-13].

In 2019, 463 million adults aged 20-79 years (8.8% of the adult population) was estimated globally to be living with DM[14]. Among these people presenting the condition, 327 million were within the working age group (20-64 years), and 136 million people were aged 65-79 years. More than four million deaths were attributed to DM in 2019, ranking it as the ninth leading cause of death globally in that year. The latest evidence shows that DM continues to be a significant global health challenge and is likely to continue to grow substantially in the next coming decades. As a result, the global epidemic of DM has key implications for healthcare costs, with most countries allocating between 5% to 20% of their total healthcare resources to treat DM and its complications[15]. Likewise, an optimal DM treatment access at lower cost would increase the success of any intervention plan, particularly in developing countries.

The emergence of the global DM epidemic can be attributed to well-documented drivers like the increasing number of older people and increasing levels of physical inactivity and obesity. However, concerns have been increased in people under the age

of 60 since more than one-third of the DM-related deaths are occurring in this age group[16]. Increased consumption of unhealthy food and sedentary lifestyle boosting an obesogenic environment have been identified as potential drivers of these growing trends[17]. Likewise, an increased risk of DM has been linked to several other sociodemographic and economic factors including age, sex, ethnicity, education, health services, employment security, housing, and access to nutritious food. For example, it has been found that in regions with increased wealth, the rates of DM increase between two and four times in individuals with low socio-economic status, potentially linked with poor access to healthy food and adequate healthcare[18,19]. Poorer individuals are less likely to be diagnosed and to get treatment early enough, resulting in earlier adverse outcomes. These socio-economic determinants can also boost the development and progression of DM through the pathways of psychological, physiological, and behavioral responses like the development of mental health conditions and chronic stress[15].

Despite significant investments in research, public health interventions, and clinical care, the slackening of the growth rate of DM has been rather modest. This epidemic will involve an urgent and steady commitment aiming to implement assertive solutions at local and national levels with public health funding oriented towards public policies and economic boosting for local communities to start DM prevention programs. However, we first must understand the global burden of DM to plan for current needs, and to inform the design and implementation of cost-effective interventions. Geographic Information Systems (GIS) methods and spatial epidemiology concepts are important tools for recognizing the spatial and temporal dynamics of the epidemic. Understanding the critical geographical characteristics of the DM epidemic will provide valuable information to identify the potential environmental, demographic, and socio-economic drivers of the epidemic as well as the geographic areas where vulnerable populations are located, and where interventions should be implemented.

The main aim of this review is to summarize the main concepts and methods used in the spatial epidemiology of DM, and to discuss the advances in the study of the spatial structure of DM epidemics. The article starts with a brief summary of the general concepts and advances in the study of the spatial dynamics of diseases followed by a summary of the current knowledge of the spatial structure of DM. The article continues with a section discussing the interaction between DM and other communicable and non-communicable diseases. It finishes with the conclusions and the advances in the study of the spatial epidemiology of DM.

SPATIAL EPIDEMIOLOGY AND GIS

Epidemiology involves the study of the distribution and determinants of diseases in populations, particularly in human populations. Epidemiologic studies have focused on identifying the type and extent of illnesses affecting human populations, and recognizing the factors linked to disease outcomes[20]. Epidemiologists examine the interactions that emerge among the host, agent, and environment (the epidemiologic triangle) to identify the underlying causes of a disease and generate interventions for prevention and control[21]. Epidemiological studies aim to explain the amount and distribution of disease within a particular population by identifying the persons at risk, the time of disease onset, and the places where they are located[22].

Traditional epidemiology has historically focused on persons and time but less on place[23]. Only recently, the geographical place at which epidemics emerge and disperse started gaining relevance as an essential element for the understanding of epidemic dynamics, and for identifying the underlying factors boosting the epidemic. As a result, spatial epidemiology has emerged as a novel approach for the understanding and control of current epidemics. In spatial epidemiology, place is a very broad concept that refers to the 'lived space' in which individuals of a given community interact. The lived space includes the natural and built environment, and human social networks and interactions. It exists in different scales, from the global and regional spaces to the individual scale[24]. Place in spatial epidemiology involves exploring beyond individual characteristics to consider the social and environmental contexts experienced by individuals and how these interactions affect their health.

The capacity to study and understand the role of places in disease dynamics has substantially increased in the past several decades. This advance is mostly linked to advances in quantitative methods and geospatial technologies such as spatial analysis and GIS. Equally important, this development is linked to new concepts and

understandings about the social conformation of places, how people interact with these social and environmental elements, and ultimately, how these interactions affect an individual's health[25]. Alongside the rapid development of geographic tools, GIS methods, and spatial analysis, massive amounts of geocoded environmental and social data are now available[26,27]. These data and the methodological tools available for analyzing spatial data, provide a strong foundation for innovative spatially explicit approaches in epidemiological research by shedding light on the etiology of health outcomes, and providing a geographical foundation for health policy-making[28]. Spatial analysis methods can be used to understand mapped information by identifying patterns and drivers of disease distribution. These methodological tools also facilitate data exploration and pattern identification by detecting unusual geographical distributions of health events and following their evolution in space over time.

Spatial epidemiology has been mostly used for studying communicable diseases, but its application has currently expanded to the study of non-communicable diseases like DM[29-32]. The quantitative methods in spatial epidemiology for estimating the net contribution of geographical hotspots (areas experiencing a disproportionately large burden of the disease) and the disease burden determinants at the ecological and individual-level may facilitate the design and implementation of control measures. The identification of these areas can uncover the locations of high-risk populations as well as revealing the factors that facilitate the persistence and spread of epidemics. In this context, spatial epidemiology has become essential tool in the fight against devastating epidemics such as malaria and HIV[33-36], but it is also becoming a widely implemented approach for the study of non-communicable diseases like cancer [37] and cardiovascular diseases[38]. Geospatial analysis has significant potential for enhancing the effectiveness of both a single program at a given level of spending and the allocation of limited resources across several programs. In sum, one of the specific implementations of spatial analysis can be to investigate how prevention and treatment interventions can be combined in different geographical places and populations for maximizing cost-effectiveness under local or national funding constraints.

SPATIAL MAPPING OF DIABETES AND DISEASE DISTRIBUTION

Disease mapping is one of the first steps for understanding the spatial structure and dynamics of a disease. Spatial maps of diseases illustrate the distribution and intensity of a disease, facilitate the identification of the heterogeneous distribution of the disease in a specific region, and elucidate high burden areas in which the disease is concentrated in space. In this context, it has been shown that the burden of DM has considerable regional variation, with developing countries being the areas disproportionately affected by the current global DM epidemic, with approximately 75% of people with DM residing in low and middle-income countries[39,40]. Meanwhile, general distribution patterns of the global DM prevalence are consistent with socio-economic development (Figure 1). Developed regions, such as Western Europe, show substantially higher DM prevalence rates that continue to increase despite the implementation of several public health measures. In 2019 the age-adjusted DM prevalence was 4.7% in Africa, 6.3% in Europe, 11.4% in the Western Pacific and Southeast Asia, 8.5% in South and Central America, 12.2% in the Middle East and North Africa, and 11.1% in North America and the Caribbean[39].

All regions in the world are expected to have an upsurge in the numbers of people living with DM. Overall, the estimated number of adults with DM will increase by 51% by 2045. While the African region currently holds the lowest prevalence of adults living with DM, it is expected to have the largest proportional increase in the numbers of adults with DM by 2045, with an increase of about 140%[41]. Moreover, the majority of people with DM in high-income countries are over the age of 50, whilst in the middle- and low-income countries are under the age of 50. The demographic patterns observed are likely to change considerably over the next generations, with improvements in life expectancy and higher rates of urbanization in these low- and middle-income countries[42]. Of notice, current general patterns indicate that there are more people living with DM in urban areas (65%) than in rural areas (35%)[15].

There is also substantial spatial variation of the distribution of DM at the subnational and local levels. To illustrate these variations, we mapped the subnational prevalence of DM in nine countries using data from the Demographic and Health Survey conducted in these countries (Figure 2). The description of the methods used

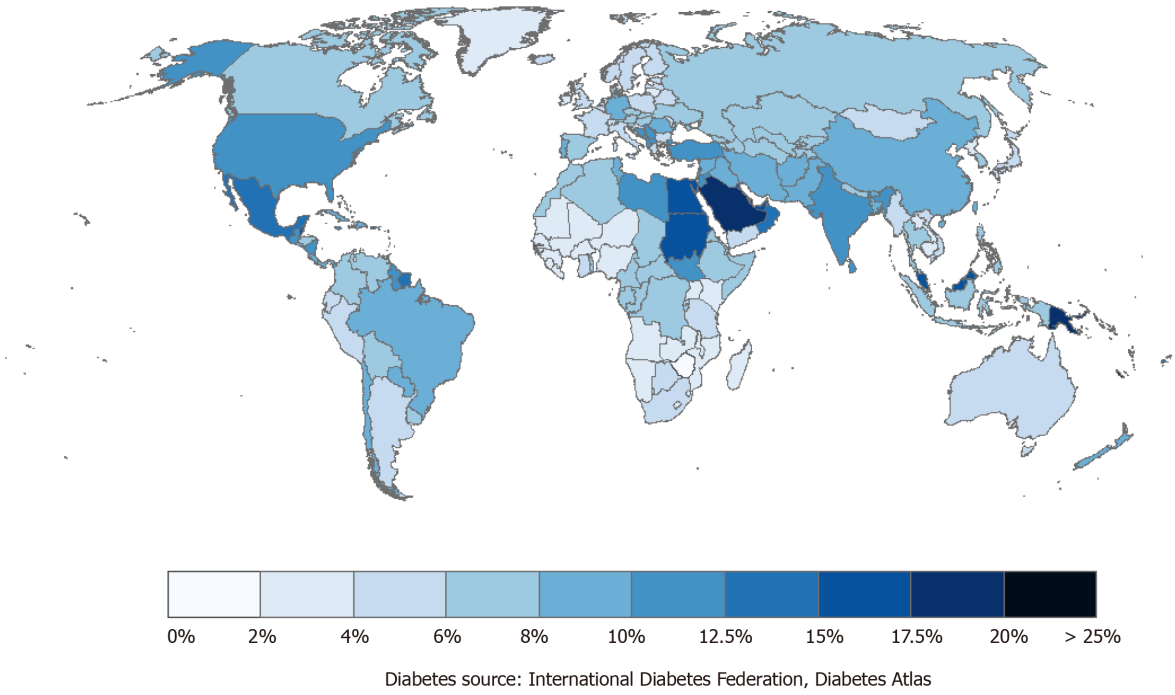

Figure 1 Global distribution of diabetes prevalence in 2017. Maps were created using ArcGIS® by ESRI version 10.5 (http://www.esri.com)[91].

for the generation of these maps can be found in Supplementary Material. The results of these maps illustrate the substantial local variation of DM prevalence within countries from different regions. The spatial structure of DM was characterized by local high DM prevalence areas where the burden of the disease was concentrated within a country (red areas in the map). These high burden areas identified locations in which the vulnerable population at high risk of the disease and the local drivers of the disease were concentrated. Such information would help to develop geographically targeted interventions tailored to the high-risk populations vulnerable to the disease at the subnational and local levels.

SPATIAL EPIDEMIOLOGY OF DIABETES

A spatial approach to disease analysis is relevant to any non-communicable disease linked to potential environmental and socio-economic drivers heterogeneously distributed in space. This approach is especially relevant to DM as recent research has identified potential associations between the spatial distribution of DM prevalence and geographical and environmental factors such as increased fast-food availability, green space, car-dominated transport, walkability and reduced spaces for exercise[43,44]. Many of these factors can be modified and included in health promotion programs, and spatial analysis can provide valuable evidence to inform resource allocation and public policy decisions targeting these potential geographical drivers of DM[45].

As mentioned previously, several demographic, clinical, and genetic factors are linked with an increased risk of developing DM[46]. In addition, there is now evidence of geographical and environmental factors associated with DM prevalence[47], and several of these factors are modifiable and candidates to be included in prevention programs, including lifestyle choices and associated cardiovascular risk, and other neighborhood factors amenable to health promotion programs. Therefore, the identification of areas where higher prevalence of environmental-related risk factors are concentrated would allow the design and implementation of geographically targeted health promotion programs oriented towards DM treatment and prevention. Moreover, spatial analysis designed to targeting health care needs at a local level can be essential in evaluating which areas are at higher risk of becoming unhealthy and thus need to be prioritized in the future by the identification of communities with a high-risk profile and the development of appropriate primary care interventions for DM prevention and treatment[48].

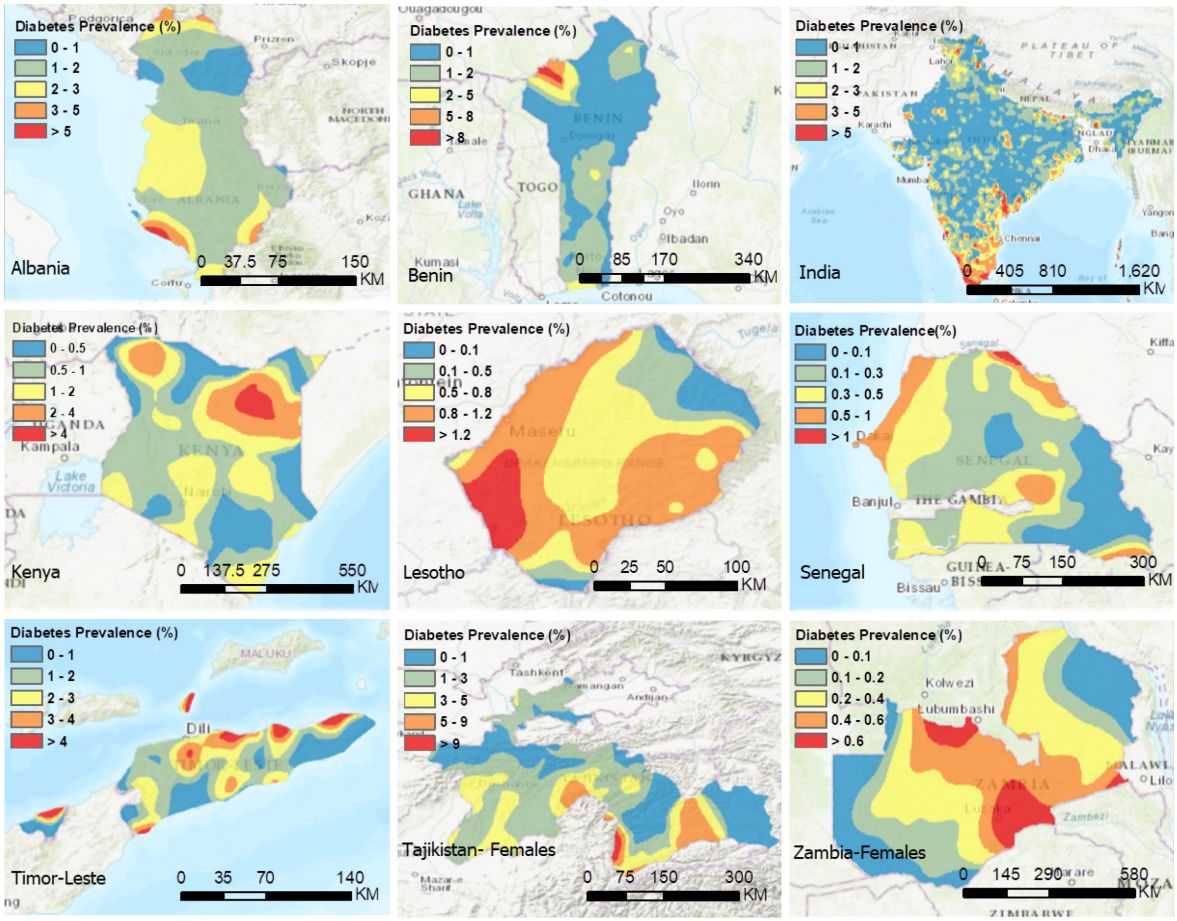

Figure 2 Spatial variations of diabetes prevalence in nine countries using a kernel smooth method. Maps were created using ArcGIS® by ESRI version 10.5 (http://www.esri.com)[91].

Several studies have identified geographical inequalities in the prevalence of risk factors and disease outcomes linked to complications of clinical DM. Disease clustering is observed not only among sociodemographic groups but also in urban geographies that boost obesity and physical inactivity[49-51]. Likewise, many built environment factors such as the rural–urban matrix[52,53], access to healthy foods[54], walkability [52], and crime levels[47] have been found to correlate with the heterogeneous distribution of DM prevalence. As the need for community-based interventions increases, it becomes essential to identify those communities at the highest risk of disease and DM complications in order to design suitable interventions based on their unique disease risk environment. Information on the relationship between DM prevalence and spatial factors such as the built environment could allow the design of better public health programs targeting populations at risk for DM. A short summary of several studies implementing different spatial methods to explore the spatial structure of DM is included in Table 1 and Supplementary Table 1.

Other recent important environmental factors have been linked with a higher risk of DM, particularly air pollution. Air pollution is an important global health problem, and $PM_{2.5}$, which is one of the most widely studied air pollutant, has been found to be associated with increased risk of several non-communicable diseases, including cardiovascular, pulmonary, kidney, and other diseases[55,56]. It has been estimated that this pollutant contributed to about 4.2 million premature deaths in 2015[57]. Growing evidence suggests a significant association between increased $PM_{2.5}$ exposure and the risk of DM, and it has been estimated that about 3.2 million cases of incident DM can be attributable to elevated concentrations of $PM_{2.5}$ in 2016[58]. Likewise, these studies have found substantial geographical variation in the burden of DM attributable to air pollution, and the link is stronger in less developed regions[58].

As countries develop economically and undertake an epidemiological transition, non-communicable diseases are likely to emerge even more prominent as major causes of disease and death, and the contribution of environmental factors including air pollution in the burden of non-communicable diseases in general, and specifically to DM, will probably become even more pronounced. The modifiable feature of these

Table 1 Methods for geospatial analysis with examples of applications and findings

Methods	Geospatial techniques	Ref.	Geographic unit and location	Key findings
	Getis-Ord Gi	[92]	West Adelaide, Australia	Spatial distribution of dementia, depression, and type 2 diabetes varied across west Adelaide, respectively. Spatial convergence of the three diseases was identified in two large hot spot clusters
Spatial clustering	Local Moran's I and the Getis-Ord Gi	[93]	Individual level in west Adelaide, Australia	Spatial heterogeneity in type 2 diabetes risk was present across communities, with significant clusters in the central part of the study area
	Moran's I	[31]	District level in India	The prevalence of diagnosed diabetes was substantially higher than that of self-reported diabetes in southern India (7.64% *vs* 2.38%) Diagnosed diabetes prevalence had positive moderate autocorrelation, and it varied from 10.52% in Goa to 4.89% in Telangana. The diagnosed diabetes prevalence was associated with higher proportion of people with secondary education and above, wealthy and Christian populations
	Moran's index and spatial regression	[94]	District level in Southern India	Spatial variations of high blood glucose (HBG) and very high blood glucose (VHBG) were observed across districts for women aged 15-49 years. District-level prevalence of HBG and VHBG were clustering across southern Indian districts. The HBG and VHBG prevalence were associated with district-level proportion of tobacco use, overweight, obese, and general caste
	Spatial statistic scan	[72]	Individual level in India	Substantial geographic variation in diabetes prevalence in India was found, with a concentrated burden at the southern coastline; Regional tuberculosis endemicity and diabetes spatial distributions showed that there is a lack of consistent geographical overlap between these 2 diseases
	Getis-Ord Gi	[95]	Individual and statistical area level 1 regions in western Adelaide, South Australia	The spatial heterogeneity of obesity, cardiovascular diseases (CVD), and type 2 diabetes was present across communities. Hot spots of these conditions clustered in three locations across western Adelaide. Area-level prevalence of CVD, obesity, and type 2 diabetes were negatively associated with socioeconomic status (SES)
	Global Moran's I, Local Moran's I and spatial regression	[32]	Individual and state level in Nigeria	Geographic clustering of diabetes mellitus (DM) and a DM pocket existed in the southeastern part of Nigeria. Obesity and education attainment were associated with the geographic variations of DM in the country
	Moran's I	[96]	Individual level in the city of Oslo, Norway	Diabetes prevalence clustered on the east side of Oslo. The diabetes prevalence was positively associated with neighborhoods with more fast foods and less healthy food shops and physical exercise facilities
	Spatial scan statistic and non-spatial linear regression	[47]	Administrative health area in the City of Winnipeg, Canada	Substantial clustering and small-area variations in DM prevalence existed in the city of Winnipeg. High rates of DM prevalence were associated with low SES, poor environmental quality and poor lifestyle
Spatial estimation models	Geographically weighted regression	[97]	Country-level of the continent United States	Significant spatial clustering of county-level diabetes prevalence was observed in the United State; the associations between diabetes prevalence and the percentage of poverty and percentage nonwhite population varied across regions in the United States.
	Geographically weighted regression	[98]	Country-level of the continent United States	The relationships between diabetes prevalence and poverty varied as a function of location
	Geographically weighted regression	[99]	Four-digit postal code level in Netherlands	Type 2 DM drug use is positively associated with population ageing, proportion of social welfare/benefits, proportion of low income, and proportion of pensioners. Spatial variabilities existed in these associations. Spatial analysis provided added value in predicting health care use at local level
	Tests of spatial autocorrelation and geographically weighted regression	[51]	Hospital referral regions in the United States	Lower-extremity amputation had spatial variations, with high rates clustered in southern states of the United States
	Spatial regression models	[100]	census-tract level in Chicago	Hypertension prevalence rates for patients were positively associated with areas with high rates of poverty, minority, and disability status. Neighboring tracts with high disease rates were the strongest predictor of cardiovascular-related chronic disease by several orders of magnitude. Diabetes had similar results
	Spatial autoregressive model	[101]	District-level in India	Spatial clustering was present in the burden of diabetes among women. The burden was relatively higher among women from the Southern and Eastern parts of the country. Diabetes was associated with obesity, hypertension, and living in urban areas
Multilevel models	Multilevel models	[102]	Individual level in Northern Netherlands	Individual risk factors at the neighborhood and municipality level explained 67.0% and 71.6% of the regional variations, respectively. Analysis on the smallest spatial scale best captured the regional variance. Individual and

				neighborhood body mass index (BMI) had significant interaction adjusting for the individual risk profile
	Multilevel negative binomial regression	[103]	Province level in China	Compared with the South, diabetes mortality was higher in the Northwest and Northeast. Diabetes mortality was higher in urbanized areas, with higher mean body mass index, and with higher average temperatures. Diabetes mortality was lower where consumption of alcohol was excessive
	Multilevel logistic regression	[104]	Province level in China	Diabetes prevalence and detection had widespread geographic variations across provinces in China. Adjusted regional diabetes prevalence was higher in the north (12.7%) than in the northeast (8.3%). Adjusted regional diabetes prevalence was higher in urban high socioeconomic circumstances (SEC) (13.1%) than in rural low-SEC counties/districts (8.7%). Adjusted diabetes detection was higher in the north (40.4%) and in urban high-SEC counties (40.8%) than in the southwest (15.6%) and the rural low-SEC counties (20.5%)
	Multilevel poisson regression	[105]	Canton-level in Southeastern France	Prevalence of treated diabetes was significantly higher in the more deprived and population-dense cantons
	Multilevel logistic regression	[106]	Census blocks in Paris, France	Prevalence of type 2 diabetes was higher in neighborhoods with the lowest levels of education attainment. Meanwhile, accounting for geographic variations in participation led to an 18% decrease in the log prevalence for low versus high neighborhood educations
Spatial analysis and GIS mapping	Choropleth mapping and logistic regression	[29]	County-level United States	Identifying a diabetes belt consisting of 644 counties in 15 mostly southern states in the United States People in the diabetes belt were more likely being Non-Hispanic African American, leading a sedentary lifestyle, and being obese
	GIS methodology of spatial join	[107]	Census-tract level in greater Sacramento area United States	Neighborhood SES was a barrier to optimal glucose control, but not associated with low-density lipoprotein control. GIS analysis is useful for disease management programs
	Data aggregation to state-level and region-level	[108]	State-level United States	The spatial variations in the ratios of children with diabetes to pediatric endocrinologists were present: the ratios in Midwest (370: 1), South (335: 1), and West (367: 1) are twice as high as in the Northeast (144: 1). Across states, there is up to a 19-fold difference in the observed ratios of obese children to pediatric endocrinologists
	Data aggregation to district level and GIS mapping	[109]	Tower Hamlets, an inner city district of London, United Kingdom	Hot spots where up to 17.3% of all adults were at high risk of developing type 2 diabetes were identified. Small-area geospatial mapping is feasible for epidemiological and environmental data
	Data aggregation to electoral wards and Regression analysis	[110]	Electoral wards in England	The diabetes prevalence varied across different locations, ranging from 2.4% in Thames Valley to 4% in North East London. The methodology of prevalence estimates is applicable to developing small area prevalence estimates for a range of chronic diseases
	Data aggregation to electoral wards and GIS mapping	[111]	Electoral wards in Greater London	Environmental factors affected diabetes outcomes. The age-adjusted mortality rates in diabetic patients were higher in deprived areas than in prosperous areas
	Data aggregation to climato-geographic and administrative regions of the Ukraine	[112]	Administrative regions in Ukraine	Geographic variations in the insulin-dependent diabetes mellitus (IDDM) were present across various administrative regions of the Ukraine. The prevalence of IDDM varied from 1740 to 3813 patients per 1 million populations across Ukraine, with the west zone having lower prevalence than the average
Bayesian estimation approaches	Bayesian spatial analysis	[113]	Local administrative district level in Bangladesh	People of older age, higher education, better socio-economic condition, higher BMI were more likely to have hypertension and diabetes. Significant regional variations were observed with prevalence for hypertension ranges between 10% and 35% and for diabetes between 6% and 19% while their national prevalence were reported as 24% and 11%, respectively
	Bayesian hierarchical joint spatial analysis	[114]	Electoral wards in the Yorkshire, United Kingdom	Childhood lymphoblastic leukemia and type 1 diabetes varied across geographic locations, clustering in more rural areas
	Bayesian Small Area Estimates	[30]	County-level United States	Diabetes incidence was high in the southeastern United States, the Appalachian region, and in scattered counties throughout the western United States
	Bayesian estimation approach	[115]	Zip code census tract of United States	Significant spatial effects existed in the diabetes prevalence even after adjusting for age, education, ethnicity and known state predictors
Regression accounting for spatial variations	Regression-based β-convergence approach, accounting for spatial autocorrelation	[116]	County-level United States	County-level disparities in diagnosed diabetes prevalence in the United States broadened, while the disparities in diagnosed diabetes incidence narrowed. Demographic, socio-economic characteristics and risk factors of type 2 diabetes were associated with changes in disparities
	Sparse Poisson convolution; sparse Poisson missing-completely-at-random	[117]	County-level; Tract-level United States	The type 1 and type 2 DM incidences in young in United States varied across regions; the type 1 and type 2 DM incidences also differed across small areas within study region. The joint spatial correlation between type 1 DM and type 2 DM was present at the county level, but not at tract level

HBG: High blood glucose; VHBG: Very high blood glucose; SES: Socioeconomic status; DM: Diabetes mellitus; GIS: Geographic Information Systems; BMI: Body mass index; IDDM: Insulin-dependent diabetes mellitus.

attributes implies that changes in these factors can yield to positive reduction in risk and might generate substantial reductions in the burden of DM in high-risk areas.

DISEASE INTERACTIONS

Interactions between diseases are recently being recognized as a key factor that influences the natural history of the diseases[59-61]. Synergistic relationships between diseases coexisting in a population could influence epidemic dynamics, resulting in marked differences in health outcomes compared to the disease occurring isolated in the population[62,63]. Therefore, focusing on the dynamics of an isolated epidemic could prevent the identification of important processes that emerge from the syndemic relationship between diseases present in the affected population[61,63]. Understanding the mechanisms that shape within-population disease clustering could be essential for the design of disease control programs[61,63]. The spatial clustering of diseases and the identification of vulnerable populations to the disease must be recognized to integrate the key social and environmental risk factors that facilitate disease interactions among vulnerable populations. In this context, spatial epidemiology and disease mapping can help to provide effective information underlining the importance of the geographical clustering of several diseases within populations, the social and environmental contexts in which diseases cluster, the ways comorbidity affects the natural history of each other, the pathways of disease interaction, and how important these interactions can be to the health burden within affected populations.

The concept of disease interaction in the epidemiology of DM has been well documented at both population and individual levels. There is substantial evidence regarding the association between DM and other macro-and microvascular diseases such as hypertension, coronary arterial disease, and congestive heart failure. Other studies have also shown a significant association between DM and several cancer conditions such as colorectal cancer, pancreatic cancer, endometrial cancer, and prostate cancer. DM has also been associated with other non-communicable diseases such as acute pancreatitis, biliary disease, psoriasis, urinary tract calculi, and chronic obstructive pulmonary disease.

While the association between DM and other non-communicable disease are well investigated and repeatedly reported in the literature, the association between DM and communicable disease have been less investigated (and only within the last few decades) mainly due to the complex pathogenesis of the association. The comorbidity of DM and tuberculosis (TB) appears to be one of the syndemics between a non-communicable and communicable disease that have reached the interest of the public health practitioners[64-67]. The association between TB and DM has been documented first for over 1000 years ago by Avicenna (980-1027 AD). With the emergence of proper diagnostics and treatments (for both TB and DM) in the second half of the 20th century, the TB-DM association, along with its possible implications, had become neglected in policy and practice[68]. In recent decades, with the increasing DM (specifically T2DM) prevalence globally, particularly in low-income countries, the relationship has re-emerged as a significant public health problem—more so in developing countries where the prevalence of DM is rising, and TB is endemic[65]. DM increases susceptibility to TB by threefold and has an adverse effect on TB treatment outcomes[64,69-71]. The combination of TB and DM represents a global health threat, specifically in any setting where the overlap of populations at risk for both diseases is increasing. Regions like Western Pacific and Africa could be most affected by such interaction (*i.e.*, number of people with TB is high and DM is increasing substantially), while regions like Europe will not be substantially affected by such interaction. The spatial interaction between DM and TB has been explored and some evidence suggests that there is a spatial association between DM and TB. However, the evidence is inconsistent and more regional research is needed in this field[72].

Moreover, hepatitis C virus (HCV) infection and DM have been shown to coexist in an individual — DM modifies the course of HCV[73-75]. Based on the currently limited evidence, studies suggest that DM is associated with increased susceptibility to HCV infection[73,76-78]. This interaction could have an impact in countries such as

Egypt in which HCV prevalence is high and number of people living with DM is and will remain high. Malaria has also been documented to be more common in DM patients, in studies from Africa, but more evidence (and stronger) are needed in this regard as well[79]. Lastly, individuals with dengue fever and DM comorbidity seem to be at higher risk of developing complications and severe dengue compared to the general population[80,81]. However, a better understanding of the relevance of comorbidities in severe dengue are needed for regions with high prevalence of dengue infection (such as tropical and subtropical areas).

Some of the more recent evidence show that DM worsen the outcomes of other viral infections such as the severe acute respiratory syndrome coronavirus or the influenza A infection[82,83]. With the emergence of the novel RNA beta coronavirus, coronavirus disease 2019 (COVID-19) globally, DM has shown to affect the development and progression of COVID-19. Published evidence, though with limitations, has already reported that DM increases the risk of COVID-19 progression and severity of disease outcome[84]. A national-representative study that analyzed 61 million medical in-hospital records in the United Kingdom, reported that one-third of COVID-19 deaths occurred in people with T2DM[85]. Adjusted for previous hospital admissions with coronary heart disease, cerebrovascular disease, or heart failure, the study showed that odds of in-hospital deaths was 2.9 for T1DM and 1.8 for T2DM[85]. Data from the United States also highlighted that DM was reported as an underlying condition for approximately 4 in 10 COVID-19 patient and that about half of people younger than 65 who died from COVID-19 had DM. Hypotheses are still being explored to understand the biological mechanism(s) behind the collision of an old metabolic disease with a new infectious disease.

CONCLUSION

With nearly 400 million people around the world having prediabetes, DM emerges as one of the biggest public health challenges of our current times. However, one of the most significant public health advances in the study of DM is the demonstration that DM can be prevented by the implementation of effective interventions targeting the factors that exacerbate the risk of DM[86-89]. Early identification of vulnerable populations at higher risk of developing DM and the location of the areas where the risk is concentrated will help policymakers to tailor and target preventative interventions to communities with the greatest need. Likewise, more studies elucidating the direct role of spatial cofactors and the prevalence of diabetes are needed. Policymakers are interested in developing community-based interventions to manage DM, hence, it is of the greatest importance that we are able to identify communities at the highest risk so that appropriate intervention programs may be designed and implemented based on the community risk profile[90]. The value of spatial analysis could be fundamental in this task by identifying high burden areas or 'hotspots' of DM risk across high-risk communities. These spatial studies can highlight regions that can benefit from strategic geographically designed interventions aimed to manage and monitor the early emergence of DM epidemics, but also can identify potential areas with high burden of other diseases, where the interaction of these diseases and DM is high.

All countries, but particularly middle-and low-income countries with constrained resources, need to thoroughly maximize the benefits and reduce costs by efficiently allocate the scarce healthcare resources for targeted interventions for DM prevention and treatment. Spatially informed tailored strategies that allocate resources to prevent and treat DM would be an effective approach aimed to control and reduce the burden of the disease. Spatially explicit community-level policy interventions addressing key factors such as healthy food supply, a friendly built environment, tax policy, financial incentives and disincentives, and disease interactions would offer great promise in effectively addressing the obesogenic and diabetogenic environment aimed to control the global DM epidemic.

REFERENCES

1 **Gabbay KH**. Hyperglycemia, polyol metabolism, and complications of diabetes mellitus. *Annu Rev Med* 1975; **26**: 521-536 [PMID: 238458 DOI: 10.1146/annurev.me.26.020175.002513]

2 **Eizirik DL**, Pasquali L, Cnop M. Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. *Nat Rev Endocrinol* 2020; **16**: 349-362 [PMID: 32398822 DOI:

10.1038/s41574-020-0355-7]
3 **World Health Organization**. Diabetes Fact sheet N°312. Updated January 2015. [cited 1 February 2021]. Available from: http://www.who.int/mediacentre/factsheets/fs312/en/
4 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013; **36** Suppl 1: S67-74 [PMID: 23264425 DOI: 10.2337/dc13-S067]
5 **American Diabetes Association**. (2) Classification and diagnosis of diabetes. *Diabetes Care* 2015; **38** Suppl: S8-S16 [PMID: 25537714 DOI: 10.2337/dc15-S005]
6 **American Diabetes Association**. 6. Glycemic Targets: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018; **41** (Suppl 1): S55-S64 [PMID: 29222377 DOI: 10.2337/dc18-S006]
7 **Minges KE**, Zimmet P, Magliano DJ, Dunstan DW, Brown A, Shaw JE. Diabetes prevalence and determinants in Indigenous Australian populations: A systematic review. *Diabetes Res Clin Pract* 2011; **93**: 139-149 [PMID: 21742398 DOI: 10.1016/j.diabres.2011.06.012]
8 **Coppell KJ**, Mann JI, Williams SM, Jo E, Drury PL, Miller JC, Parnell WR. Prevalence of diagnosed and undiagnosed diabetes and prediabetes in New Zealand: findings from the 2008/09 Adult Nutrition Survey. *N Z Med J* 2013; **126**: 23-42 [PMID: 23474511]
9 **Jørgensen ME**, Bjerregaard P, Borch-Johnsen K, Witte D. New diagnostic criteria for diabetes: is the change from glucose to HbA1c possible in all populations? *J Clin Endocrinol Metab* 2010; **95**: E333-6 [PMID: 20739381 DOI: 10.1210/jc.2010-0710]
10 **Fletcher B**, Gulanick M, Lamendola C. Risk factors for type 2 diabetes mellitus. *J Cardiovasc Nurs* 2002; **16**: 17-23 [PMID: 11800065 DOI: 10.1097/00005082-200201000-00003]
11 **Sullivan PW**, Morrato EH, Ghushchyan V, Wyatt HR, Hill JO. Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. *Diabetes Care* 2005; **28**: 1599-1603 [PMID: 15983307 DOI: 10.2337/diacare.28.7.1599]
12 **Rajagopalan S**, Brook RD. Air pollution and type 2 diabetes: mechanistic insights. *Diabetes* 2012; **61**: 3037-3045 [PMID: 23172950 DOI: 10.2337/db12-0190]
13 **Tobias DK**, Hu FB, Chavarro J, Rosner B, Mozaffarian D, Zhang C. Healthful dietary patterns and type 2 diabetes mellitus risk among women with a history of gestational diabetes mellitus. *Arch Intern Med* 2012; **172**: 1566-1572 [PMID: 22987062 DOI: 10.1001/archinternmed.2012.3747]
14 **Saeedi P**, Salpea P, Karuranga S, Petersohn I, Malanda B, Gregg EW, Unwin N, Wild SH, Williams R. Mortality attributable to diabetes in 20-79 years old adults, 2019 estimates: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2020; **162**: 108086 [PMID: 32068099 DOI: 10.1016/j.diabres.2020.108086]
15 **Dagogo-Jack S**. Diabetes mellitus in developing countries and underserved communities. Springer, 2017
16 **Alotaibi A**, Perry L, Gholizadeh L, Al-Ganmi A. Incidence and prevalence rates of diabetes mellitus in Saudi Arabia: An overview. *J Epidemiol Glob Health* 2017; **7**: 211-218 [PMID: 29110860 DOI: 10.1016/j.jegh.2017.10.001]
17 **Lone S**, Lone K, Khan S, Pampori RA. Assessment of metabolic syndrome in Kashmiri population with type 2 diabetes employing the standard criteria's given by WHO, NCEPATP III and IDF. *J Epidemiol Glob Health* 2017; **7**: 235-239 [PMID: 29110863 DOI: 10.1016/j.jegh.2017.07.004]
18 **Tuomilehto J**, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-1350 [PMID: 11333990 DOI: 10.1056/NEJM200105033441801]
19 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527 DOI: 10.1056/NEJMoa012512]
20 **Krieger N**. Epidemiology and the people's health: theory and context. Oxford University Press, 2011
21 **McAnally HB**. Opioid dependence: a clinical and epidemiologic approach. Springer, 2017
22 **Rothman KJ**, Greenland S, Lash TL. Modern epidemiology. Lippincott Williams & Wilkins, 2008
23 **Moore DA**, Carpenter TE. Spatial analytical methods and geographic information systems: use in health research and epidemiology. *Epidemiol Rev* 1999; **21**: 143-161 [PMID: 10682254 DOI: 10.1093/oxfordjournals.epirev.a017993]
24 **Kearns RA**, Joseph AE. Space in its place: developing the link in medical geography. *Soc Sci Med* 1993; **37**: 711-717 [PMID: 8211286 DOI: 10.1016/0277-9536(93)90364-a]
25 **Rushton G**. Public health, GIS, and spatial analytic tools. *Annu Rev Public Health* 2003; **24**: 43-56 [PMID: 12471269 DOI: 10.1146/annurev.publhealth.24.012902.140843]
26 **Nykiforuk CI**, Flaman LM. Geographic information systems (GIS) for Health Promotion and Public Health: a review. *Health Promot Pract* 2011; **12**: 63-73 [PMID: 19546198 DOI: 10.1177/1524839909334624]
27 **Thomas YF**, Richardson D, Cheung I. Geography and drug addiction. Springer Science & Business Media, 2008
28 **McLafferty SL**. GIS and health care. *Annu Rev Public Health* 2003; **24**: 25-42 [PMID: 12668754 DOI: 10.1146/annurev.publhealth.24.012902.141012]
29 **Barker LE**, Kirtland KA, Gregg EW, Geiss LS, Thompson TJ. Geographic distribution of diagnosed diabetes in the U.S.: a diabetes belt. *Am J Prev Med* 2011; **40**: 434-439 [PMID:

21406277 DOI: 10.1016/j.amepre.2010.12.019]
30 **Barker LE**, Thompson TJ, Kirtland KA, Boyle JP, Geiss LS, McCauley MM, Albright AL. Bayesian Small Area Estimates of Diabetes Incidence by United States County, 2009. *J Data Sci* 2013; **11**: 269-280 [PMID: 26279666]
31 **Barua S**, Saikia N, Sk R. Spatial pattern and determinants of diagnosed diabetes in southern India: evidence from a 2012-13 population-based survey. *J Biosoc Sci* 2020; **10**: 1-16 [PMID: 32772932 DOI: 10.1017/S0021932020000449]
32 **Osayomi T**. The emergence of a diabetes pocket in Nigeria: the result of a spatial analysis. *GeoJournal* 2019; **84**: 1149-1164 [DOI: 10.1007/s10708-018-9911-2]
33 **Hay SI**, Snow RW. The malaria Atlas Project: developing global maps of malaria risk. *PLoS Med* 2006; **3**: e473 [PMID: 17147467 DOI: 10.1371/journal.pmed.0030473]
34 **Wilson D**, Halperin DT. "Know your epidemic, know your response": a useful approach, if we get it right. *Lancet* 2008; **372**: 423-426 [PMID: 18687462 DOI: 10.1016/S0140-6736(08)60883-1]
35 **Cuadros DF**, Li J, Branscum AJ, Akullian A, Jia P, Mziray EN, Tanser F. Mapping the spatial variability of HIV infection in Sub-Saharan Africa: Effective information for localized HIV prevention and control. *Sci Rep* 2017; **7**: 9093 [PMID: 28831171 DOI: 10.1038/s41598-017-09464-y]
36 **Dwyer-Lindgren L**, Cork MA, Sligar A, Steuben KM, Wilson KF, Provost NR, Mayala BK, VanderHeide JD, Collison ML, Hall JB, Biehl MH, Carter A, Frank T, Douwes-Schultz D, Burstein R, Casey DC, Deshpande A, Earl L, El Bcheraoui C, Farag TH, Henry NJ, Kinyoki D, Marczak LB, Nixon MR, Osgood-Zimmerman A, Pigott D, Reiner RC Jr, Ross JM, Schaeffer LE, Smith DL, Davis Weaver N, Wiens KE, Eaton JW, Justman JE, Opio A, Sartorius B, Tanser F, Wabiri N, Piot P, Murray CJL, Hay SI. Mapping HIV prevalence in sub-Saharan Africa between 2000 and 2017. *Nature* 2019; **570**: 189-193 [PMID: 31092927 DOI: 10.1038/s41586-019-1200-9]
37 **Roquette R**, Painho M, Nunes B. Spatial epidemiology of cancer: a review of data sources, methods and risk factors. *Geospat Health* 2017; **12**: 504 [PMID: 28555468 DOI: 10.4081/gh.2017.504]
38 **Mena C**, Sepúlveda C, Fuentes E, Ormazábal Y, Palomo I. Spatial analysis for the epidemiological study of cardiovascular diseases: A systematic literature search. *Geospat Health* 2018; **13**: 587 [PMID: 29772872 DOI: 10.4081/gh.2018.587]
39 **Ogurtsova K**, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017; **128**: 40-50 [PMID: 28437734 DOI: 10.1016/j.diabres.2017.03.024]
40 **Dunachie S**, Chamnan P. The double burden of diabetes and global infection in low and middle-income countries. *Trans R Soc Trop Med Hyg* 2019; **113**: 56-64 [PMID: 30517697 DOI: 10.1093/trstmh/try124]
41 **Guariguata L**, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014; **103**: 137-149 [PMID: 24630390 DOI: 10.1016/j.diabres.2013.11.002]
42 **Khan MAB**, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health* 2020; **10**: 107-111 [PMID: 32175717 DOI: 10.2991/jegh.k.191028.001]
43 **Kent J**, Thompson S, Jalaludin B. Healthy Built Environments: A review of the literature. *Healthy Built Environ Program* 2011
44 **Astell-Burt T**, Feng X, Kolt GS. Is neighborhood green space associated with a lower risk of type 2 diabetes? *Diabetes Care* 2014; **37**: 197-201 [PMID: 24026544 DOI: 10.2337/dc13-1325]
45 **Earnest A**, Morgan G, Mengersen K, Ryan L, Summerhayes R, Beard J. Evaluating the effect of neighbourhood weight matrices on smoothing properties of Conditional Autoregressive (CAR) models. *Int J Health Geogr* 2007; **6**: 54 [PMID: 18045503 DOI: 10.1186/1476-072X-6-54]
46 **Baker J**, White N, Mengersen K. Spatial modelling of type II diabetes outcomes: a systematic review of approaches used. *R Soc Open Sci* 2015; **2**: 140460 [PMID: 26543572 DOI: 10.1098/rsos.140460]
47 **Green C**, Hoppa RD, Young TK, Blanchard JF. Geographic analysis of diabetes prevalence in an urban area. *Soc Sci Med* 2003; **57**: 551-560 [PMID: 12791496 DOI: 10.1016/s0277-9536(02)00380-5]
48 **Chen SE**, Florax RJ. Zoning for health: the obesity epidemic and opportunities for local policy intervention. *J Nutr* 2010; **140**: 1181-1184 [PMID: 20392881 DOI: 10.3945/jn.109.111336]
49 **Peters AL**, Legorreta AP, Ossorio RC, Davidson MB. Quality of outpatient care provided to diabetic patients. A health maintenance organization experience. *Diabetes Care* 1996; **19**: 601-606 [PMID: 8725859 DOI: 10.2337/diacare.19.6.601]
50 **Yan G**, Cheung AK, Ma JZ, Yu AJ, Greene T, Oliver MN, Yu W, Norris KC. The associations between race and geographic area and quality-of-care indicators in patients approaching ESRD. *Clin J Am Soc Nephrol* 2013; **8**: 610-618 [PMID: 23493380 DOI: 10.2215/CJN.07780812]
51 **Margolis DJ**, Hoffstad O, Nafash J, Leonard CE, Freeman CP, Hennessy S, Wiebe DJ. Location, location, location: geographic clustering of lower-extremity amputation among Medicare beneficiaries with diabetes. *Diabetes Care* 2011; **34**: 2363-2367 [PMID: 21933906 DOI: 10.2337/dc11-0807]
52 **Hu FB**, Sigal RJ, Rich-Edwards JW, Colditz GA, Solomon CG, Willett WC, Speizer FE, Manson JE. Walking compared with vigorous physical activity and risk of type 2 diabetes in women: a

prospective study. *JAMA* 1999; **282**: 1433-1439 [PMID: 10535433 DOI: 10.1001/jama.282.15.1433]

53 **Ranta J**, Penttinen A. Probabilistic small area risk assessment using GIS-based data: a case study on Finnish childhood diabetes. Geographic information systems. *Stat Med* 2000; **19**: 2345-2359 [PMID: 10960858 DOI: 10.1002/1097-0258(20000915/30)19:17/18<2345::aid-sim574>3.0.co;2-g]

54 **Salois MJ**. Obesity and diabetes, the built environment, and the 'local' food economy in the United States, 2007. *Econ Hum Biol* 2012; **10**: 35-42 [PMID: 21561816 DOI: 10.1016/j.ehb.2011.04.001]

55 **Bowe B**, Xie Y, Li T, Yan Y, Xian H, Al-Aly Z. Particulate Matter Air Pollution and the Risk of Incident CKD and Progression to ESRD. *J Am Soc Nephrol* 2018; **29**: 218-230 [PMID: 28935655 DOI: 10.1681/ASN.2017030253]

56 **Bowe B**, Xie Y, Li T, Yan Y, Xian H, Al-Aly Z. Associations of ambient coarse particulate matter, nitrogen dioxide, and carbon monoxide with the risk of kidney disease: a cohort study. *Lancet Planet Health* 2017; **1**: e267-e276 [PMID: 29851625 DOI: 10.1016/S2542-5196(17)30117-1]

57 **Cohen AJ**, Brauer M, Burnett R, Anderson HR, Frostad J, Estep K, Balakrishnan K, Brunekreef B, Dandona L, Dandona R, Feigin V, Freedman G, Hubbell B, Jobling A, Kan H, Knibbs L, Liu Y, Martin R, Morawska L, Pope CA 3rd, Shin H, Straif K, Shaddick G, Thomas M, van Dingenen R, van Donkelaar A, Vos T, Murray CJL, Forouzanfar MH. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *Lancet* 2017; **389**: 1907-1918 [PMID: 28408086 DOI: 10.1016/S0140-6736(17)30505-6]

58 **Bowe B**, Xie Y, Li T, Yan Y, Xian H, Al-Aly Z. The 2016 global and national burden of diabetes mellitus attributable to $PM_{2.5}$ air pollution. *Lancet Planet Health* 2018; **2**: e301-e312 [PMID: 30074893 DOI: 10.1016/S2542-5196(18)30140-2]

59 **Telfer S**, Lambin X, Birtles R, Beldomenico P, Burthe S, Paterson S, Begon M. Species interactions in a parasite community drive infection risk in a wildlife population. *Science* 2010; **330**: 243-246 [PMID: 20929776 DOI: 10.1126/science.1190333]

60 **Pedersen AB**, Fenton A. Emphasizing the ecology in parasite community ecology. *Trends Ecol Evol* 2007; **22**: 133-139 [PMID: 17137676 DOI: 10.1016/j.tree.2006.11.005]

61 **Johnson PT**, de Roode JC, Fenton A. Why infectious disease research needs community ecology. *Science* 2015; **349**: 1259504 [PMID: 26339035 DOI: 10.1126/science.1259504]

62 **Cox FE**. Concomitant infections, parasites and immune responses. *Parasitology* 2001; **122** Suppl: S23-S38 [PMID: 11442193 DOI: 10.1017/s003118200001698x]

63 **Petney TN**, Andrews RH. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *Int J Parasitol* 1998; **28**: 377-393 [PMID: 9559357 DOI: 10.1016/s0020-7519(97)00189-6]

64 **Jeon CY**, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. *PLoS Med* 2008; **5**: e152 [PMID: 18630984 DOI: 10.1371/journal.pmed.0050152]

65 **Dooley KE**, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. *Lancet Infect Dis* 2009; **9**: 737-746 [PMID: 19926034 DOI: 10.1016/S1473-3099(09)70282-8]

66 **Ruslami R**, Aarnoutse RE, Alisjahbana B, van der Ven AJ, van Crevel R. Implications of the global increase of diabetes for tuberculosis control and patient care. *Trop Med Int Health* 2010; **15**: 1289-1299 [PMID: 20955495 DOI: 10.1111/j.1365-3156.2010.02625.x]

67 **Martinez N**, Kornfeld H. Diabetes and immunity to tuberculosis. *Eur J Immunol* 2014; **44**: 617-626 [PMID: 24448841 DOI: 10.1002/eji.201344301]

68 **Restrepo BI**. Convergence of the tuberculosis and diabetes epidemics: renewal of old acquaintances. *Clin Infect Dis* 2007; **45**: 436-438 [PMID: 17638190 DOI: 10.1086/519939]

69 **Baker MA**, Harries AD, Jeon CY, Hart JE, Kapur A, Lönnroth K, Ottmani SE, Goonesekera SD, Murray MB. The impact of diabetes on tuberculosis treatment outcomes: a systematic review. *BMC Med* 2011; **9**: 81 [PMID: 21722362 DOI: 10.1186/1741-7015-9-81]

70 **Huangfu P**, Pearson F, Ugarte-Gil C, Critchley J. Diabetes and poor tuberculosis treatment outcomes: issues and implications in data interpretation and analysis. *Int J Tuberc Lung Dis* 2017; **21**: 1214-1219 [PMID: 29297440 DOI: 10.5588/ijtld.17.0211]

71 **Al-Rifai RH**, Pearson F, Critchley JA, Abu-Raddad LJ. Association between diabetes mellitus and active tuberculosis: A systematic review and meta-analysis. *PLoS One* 2017; **12**: e0187967 [PMID: 29161276 DOI: 10.1371/journal.pone.0187967]

72 **Hernandez AM**, Jia P, Kim HY, Cuadros DF. Geographic Variation and Associated Covariates of Diabetes Prevalence in India. *JAMA Netw Open* 2020; **3**: e203865 [PMID: 32356884 DOI: 10.1001/jamanetworkopen.2020.3865]

73 **Guo X**, Jin M, Yang M, Liu K, Li JW. Type 2 diabetes mellitus and the risk of hepatitis C virus infection: a systematic review. *Sci Rep* 2013; **3**: 2981 [PMID: 25671325 DOI: 10.1038/srep02981]

74 **Elhawary EI**, Mahmoud GF, El-Daly MA, Mekky FA, Esmat GG, Abdel-Hamid M. Association of HCV with diabetes mellitus: an Egyptian case-control study. *Virol J* 2011; **8**: 367 [PMID: 21791087 DOI: 10.1186/1743-422X-8-367]

75 **Cuadros DF**, Miller FD, Nagelkerke N, Abu-Raddad LJ. Association between HCV infection and diabetes type 2 in Egypt: is it time to split up? *Ann Epidemiol* 2015; **25**: 918-923 [PMID: 26499381 DOI: 10.1016/j.annepidem.2015.09.005]

76 **Kaneko Y**, Takahashi M, Taneichi H, Sawada A, Suzuki K. [Hepatitis C and diabetes mellitus]. *Nihon Rinsho* 2002; **60** Suppl 10: 633-636 [PMID: 12430294]

77 **Bahtiyar G**, Shin JJ, Aytaman A, Sowers JR, McFarlane SI. Association of diabetes and hepatitis C infection: epidemiologic evidence and pathophysiologic insights. *Curr Diab Rep* 2004; **4**: 194-198 [PMID: 15132884 DOI: 10.1007/s11892-004-0023-7]

78 **Ratziu V**, Heurtier A, Bonyhay L, Poynard T, Giral P. Review article: an unexpected virus-host interaction--the hepatitis C virus-diabetes link. *Aliment Pharmacol Ther* 2005; **22** Suppl 2: 56-60 [PMID: 16225475 DOI: 10.1111/j.1365-2036.2005.02598.x]

79 **Kalra S**, Khandelwal D, Singla R, Aggarwal S, Dutta D. Malaria and diabetes. *J Pak Med Assoc* 2017; **67**: 810-813 [PMID: 28507380]

80 **Toledo J**, George L, Martinez E, Lazaro A, Han WW, Coelho GE, Runge Ranzinger S, Horstick O. Relevance of Non-communicable Comorbidities for the Development of the Severe Forms of Dengue: A Systematic Literature Review. *PLoS Negl Trop Dis* 2016; **10**: e0004284 [PMID: 26727113 DOI: 10.1371/journal.pntd.0004284]

81 **Htun NS**, Odermatt P, Eze IC, Boillat-Blanco N, D'Acremont V, Probst-Hensch N. Is diabetes a risk factor for a severe clinical presentation of dengue? *PLoS Negl Trop Dis* 2015; **9**: e0003741 [PMID: 25909658 DOI: 10.1371/journal.pntd.0003741]

82 **Booth CM**, Matukas LM, Tomlinson GA, Rachlis AR, Rose DB, Dwosh HA, Walmsley SL, Mazzulli T, Avendano M, Derkach P, Ephtimios IE, Kitai I, Mederski BD, Shadowitz SB, Gold WL, Hawryluck LA, Rea E, Chenkin JS, Cescon DW, Poutanen SM, Detsky AS. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 2003; **289**: 2801-2809 [PMID: 12734147 DOI: 10.1001/jama.289.21.JOC30885]

83 **Allard R**, Leclerc P, Tremblay C, Tannenbaum TN. Diabetes and the severity of pandemic influenza A (H1N1) infection. *Diabetes Care* 2010; **33**: 1491-1493 [PMID: 20587722 DOI: 10.2337/dc09-2215]

84 **Maddaloni E**, Buzzetti R. Covid-19 and diabetes mellitus: unveiling the interaction of two pandemics. *Diabetes Metab Res Rev* 2020; e33213321 [PMID: 32233018 DOI: 10.1002/dmrr.3321]

85 **Barron E**, Bakhai C, Kar P, Weaver A, Bradley D, Ismail H, Knighton P, Holman N, Khunti K, Sattar N, Wareham NJ, Young B, Valabhji J. Associations of type 1 and type 2 diabetes with COVID-19-related mortality in England: a whole-population study. *Lancet Diabetes Endocrinol* 2020; **8**: 813-822 [PMID: 32798472 DOI: 10.1016/S2213-8587(20)30272-2]

86 **Ratner RE**; Diabetes Prevention Program Research. An update on the Diabetes Prevention Program. *Endocr Pract* 2006; **12** Suppl 1: 20-24 [PMID: 16627375 DOI: 10.4158/EP.12.S1.20]

87 **LaMonte MJ**, Blair SN, Church TS. Physical activity and diabetes prevention. *J Appl Physiol (1985)* 2005; **99**: 1205-1213 [PMID: 16103523 DOI: 10.1152/japplphysiol.00193.2005]

88 **Ackermann RT**, Finch EA, Brizendine E, Zhou H, Marrero DG. Translating the Diabetes Prevention Program into the community. The DEPLOY Pilot Study. *Am J Prev Med* 2008; **35**: 357-363 [PMID: 18779029 DOI: 10.1016/j.amepre.2008.06.035]

89 **Diabetes Prevention Program (DPP) Research Group**. The Diabetes Prevention Program (DPP): description of lifestyle intervention. *Diabetes Care* 2002; **25**: 2165-2171 [PMID: 12453955 DOI: 10.2337/diacare.25.12.2165]

90 **Cravey AJ**, Washburn SA, Gesler WM, Arcury TA, Skelly AH. Developing socio-spatial knowledge networks: a qualitative methodology for chronic disease prevention. *Soc Sci Med* 2001; **52**: 1763-1775 [PMID: 11352404 DOI: 10.1016/s0277-9536(00)00295-1]

91 **ESRI**. ArcGIS 10.x. Redlands, CA, USA: ESRI. 2004. Available from: http://www.esri.com

92 **Aw JYH**, Smurthwaite K, Bagheri N. Investigating spatial convergence of diagnosed dementia, depression and type 2 diabetes prevalence in West Adelaide, Australia. *J Affect Disord* 2020; **277**: 524-530 [PMID: 32882510 DOI: 10.1016/j.jad.2020.08.032]

93 **Bagheri N**, Konings P, Wangdi K, Parkinson A, Mazumdar S, Sturgiss E, Lal A, Douglas K, Glasgow N. Identifying hotspots of type 2 diabetes risk using general practice data and geospatial analysis: an approach to inform policy and practice. *Aust J Prim Health* 2019 [PMID: 31751519 DOI: 10.1071/PY19043]

94 **Biradar RA**, Singh DP. Spatial clustering of diabetes among reproductive age women and its spatial determinants at the district level in southern India. *Clin Epidemiol Glob Health* 2020; **8**: 791-796 [DOI: 10.1016/j.cegh.2020.02.001]

95 **Smurthwaite K**, Bagheri N. Using Geographical Convergence of Obesity, Cardiovascular Disease, and Type 2 Diabetes at the Neighborhood Level to Inform Policy and Practice. *Prev Chronic Dis* 2017; **14**: E91 [PMID: 29023234 DOI: 10.5888/pcd14.170170]

96 **Nosrati E**, Jenum AK, Tran AT, Marmot SM, Peter King L. Ethnicity and place: the geography of diabetes inequalities under a strong welfare state. *Eur J Public Health* 2018; **28**: 30-34 [PMID: 29040545 DOI: 10.1093/eurpub/ckx119]

97 **Hipp JA**, Chalise N. Spatial analysis and correlates of county-level diabetes prevalence, 2009-2010. *Prev Chronic Dis* 2015; **12**: E08 [PMID: 25611797 DOI: 10.5888/pcd12.140404]

98 **Siordia C**, Saenz J, Tom SE. An Introduction to Macro- Level Spatial Nonstationarity: a Geographically Weighted Regression Analysis of Diabetes and Poverty. *Human Geogr* 2012; **6**: 5-13 [PMID: 25414731 DOI: 10.5719/hgeo.2012.62.5]

99 **Dijkstra A**, Janssen F, De Bakker M, Bos J, Lub R, Van Wissen LJ, Hak E. Using spatial analysis to predict health care use at the local level: a case study of type 2 diabetes medication use and its association with demographic change and socioeconomic status. *PLoS One* 2013; **8**: e72730 [PMID: 24023636 DOI: 10.1371/journal.pone.0072730]

100 **Kolak M**, Chua R, Kamber I, Tung EL, Besser S, Anyanwu E, Liao J, Anselin L, Tabit C. Social determinants of health and clinical hypertension and diabetes prevalence on Chicago's south side. *J Am Coll Cardiol* 2019; **73**: 1824-1824

101 **Singh S**, Puri P, Subramanian SV. Identifying spatial variation in the burden of diabetes among women across 640 districts in India: a cross-sectional study. *J Diabetes Metab Disord* 2020; **19**: 523-533 [PMID: 32550205 DOI: 10.1007/s40200-020-00545-w]

102 **Dekker LH**, Rijnks RH, Navis GJ. Regional variation in type 2 diabetes: evidence from 137 820 adults on the role of neighbourhood body mass index. *Eur J Public Health* 2020; **30**: 189-194 [PMID: 31114865 DOI: 10.1093/eurpub/ckz085]

103 **Zhou M**, Astell-Burt T, Bi Y, Feng X, Jiang Y, Li Y, Page A, Wang L, Xu Y, Zhao W, Ning G. Geographical variation in diabetes prevalence and detection in china: multilevel spatial analysis of 98,058 adults. *Diabetes Care* 2015; **38**: 72-81 [PMID: 25352654 DOI: 10.2337/dc14-1100]

104 **Zhou M**, Astell-Burt T, Yin P, Feng X, Page A, Liu Y, Liu J, Li Y, Liu S, Wang L. Spatiotemporal variation in diabetes mortality in China: multilevel evidence from 2006 and 2012. *BMC Public Health* 2015; **15**: 633 [PMID: 26159911 DOI: 10.1186/s12889-015-1982-0]

105 **Bocquier A**, Cortaredona S, Nauleau S, Jardin M, Verger P. Prevalence of treated diabetes: Geographical variations at the small-area level and their association with area-level characteristics. A multilevel analysis in Southeastern France. *Diabetes Metab* 2011; **37**: 39-46 [PMID: 20980182 DOI: 10.1016/j.diabet.2010.07.004]

106 **Chaix B**, Billaudeau N, Thomas F, Havard S, Evans D, Kestens Y, Bean K. Neighborhood effects on health: correcting bias from neighborhood effects on participation. *Epidemiology* 2011; **22**: 18-26 [PMID: 21150351 DOI: 10.1097/EDE.0b013e3181fd2961]

107 **Geraghty EM**, Balsbaugh T, Nuovo J, Tandon S. Using Geographic Information Systems (GIS) to assess outcome disparities in patients with type 2 diabetes and hyperlipidemia. *J Am Board Fam Med* 2010; **23**: 88-96 [PMID: 20051547 DOI: 10.3122/jabfm.2010.01.090149]

108 **Lee JM**, Davis MM, Menon RK, Freed GL. Geographic distribution of childhood diabetes and obesity relative to the supply of pediatric endocrinologists in the United States. *J Pediatr* 2008; **152**: 331-336 [PMID: 18280836 DOI: 10.1016/j.jpeds.2007.08.037]

109 **Noble D**, Smith D, Mathur R, Robson J, Greenhalgh T. Feasibility study of geospatial mapping of chronic disease risk to inform public health commissioning. *BMJ Open* 2012; **2**: e000711 [PMID: 22337817 DOI: 10.1136/bmjopen-2011-000711]

110 **Congdon P**. Estimating diabetes prevalence by small area in England. *J Public Health (Oxf)* 2006; **28**: 71-81 [PMID: 16436450 DOI: 10.1093/pubmed/fdi068]

111 **Weng C**, Coppini DV, Sönksen PH. Geographic and social factors are related to increased morbidity and mortality rates in diabetic patients. *Diabet Med* 2000; **17**: 612-617 [PMID: 11073184 DOI: 10.1046/j.1464-5491.2000.00352.x]

112 **Kravchenko VI**, Tronko ND, Pankiv VI, Venzilovich YuM, Prudius FG. Prevalence of diabetes mellitus and its complications in the Ukraine. *Diabetes Res Clin Pract* 1996; **34** Suppl: S73-S78 [PMID: 9015673 DOI: 10.1016/s0168-8227(96)90011-x]

113 **Roy PK**, Khan MHR, Akter T, Rahman MS. Exploring socio-demographic-and geographical-variations in prevalence of diabetes and hypertension in Bangladesh: Bayesian spatial analysis of national health survey data. *Spat Spatiotemporal Epidemiol* 2019; **29**: 71-83 [PMID: 31128633 DOI: 10.1016/j.sste.2019.03.003]

114 **Manda SO**, Feltbower RG, Gilthorpe MS. Investigating spatio-temporal similarities in the epidemiology of childhood leukaemia and diabetes. *Eur J Epidemiol* 2009; **24**: 743-752 [PMID: 19784553 DOI: 10.1007/s10654-009-9391-2]

115 **Congdon P**, Lloyd P. Estimating small area diabetes prevalence in the US using the behavioral risk factor surveillance system. *J Data Sci* 2010; **8**: 235-252

116 **Shrestha SS**, Thompson TJ, Kirtland KA, Gregg EW, Beckles GL, Luman ET, Barker LE, Geiss LS. Changes in Disparity in County-Level Diagnosed Diabetes Prevalence and Incidence in the United States, between 2004 and 2012. *PLoS One* 2016; **11**: e0159876 [PMID: 27487006 DOI: 10.1371/journal.pone.0159876]

117 **Liese AD**, Lawson A, Song HR, Hibbert JD, Porter DE, Nichols M, Lamichhane AP, Dabelea D, Mayer-Davis EJ, Standiford D, Liu L, Hamman RF, D'Agostino RB Jr. Evaluating geographic variation in type 1 and type 2 diabetes mellitus incidence in youth in four US regions. *Health Place* 2010; **16**: 547-556 [PMID: 20129809 DOI: 10.1016/j.healthplace.2009.12.015]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1057-1069

DOI: 10.4239/wjd.v12.i7.1057

ISSN 1948-9358 (online)

REVIEW

Comprehensive overview of human serum albumin glycation in diabetes mellitus

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Author contributions: Qiu HY and Hou NN were responsible for conceptualization, methodology, data curation, and original draft preparation; Shi JF, Liu YP, Kan CX, and Han F were responsible for data curation and investigation; Sun XD was responsible for supervision, manuscript writing and editing, and funding acquisition.

Supported by the National Natural Science Foundation of China, No. 81870593; Natural Science Foundation of Shandong Province of China, No. ZR2020MH106; and Medical Health Science and Technology Project of Shandong Province, No. 202003060400.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

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Abstract

The presence of excess glucose in blood is regarded as a sweet hurt for patients with diabetes. Human serum albumin (HSA) is the most abundant protein in human plasma, which undergoes severe non-enzymatic glycation with glucose in patients with diabetes; this modifies the structure and function of HSA. Furthermore, the advanced glycation end products produced by glycated HSA can cause pathological damage to the human body through various signaling pathways, eventually leading to complications of diabetes. Many potential glycation sites on HSA have different degrees of sensitivity to glucose concentration. This review provides a comprehensive assessment of the *in vivo* glycation sites of HSA; it also discusses the effects of glycation on the structure and function of HSA. Moreover, it addresses the relationship between HSA glycation and diabetes complications. Finally, it focuses on the value of non-enzymatic glycation of HSA in diabetes-related clinical applications.

Key Words: Diabetes mellitus; Human serum albumin; Non-enzymatic glycation; Advanced glycation end products; Glycation sites; Diabetic complications

Core Tip: In the case of hyperglycemia state, the glycation level of albumin in plasma is significantly increased, which alters the structure and function of albumin. Herein we review the different glycation sites and functional changes of glycated albumin, and discuss the relationship between albumin glycation and diabetes complications. The

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 16, 2021
Peer-review started: March 16, 2021
First decision: May 3, 2021
Revised: May 6, 2021
Accepted: June 4, 2021
Article in press: June 4, 2021
Published online: July 15, 2021

P-Reviewer: Ciarambino T, Gracia-Ramos AE, Lee KS
S-Editor: Zhang L
L-Editor: Wang TQ
P-Editor: Wang LL

potential application value of glycated albumin in clinical is also discussed.

Citation: Qiu HY, Hou NN, Shi JF, Liu YP, Kan CX, Han F, Sun XD. Comprehensive overview of human serum albumin glycation in diabetes mellitus. *World J Diabetes* 2021; 12(7): 1057-1069
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1057.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1057

INTRODUCTION

Diabetes is a metabolic disease caused by an absolute or relative deficiency of insulin in the human body related to various pathogenic etiologies; it leads to metabolic disorders involving sugars, lipids, and proteins, with severe hyperglycemia as the main clinical manifestation[1,2]. Abnormally high glucose concentrations in patients with diabetes can cause proteins in the body to undergo non-enzymatic glycation (*i.e.*, without the involvement of glycosyltransferase), which is the initiating factor of diabetes-related complications[3,4]. Human serum albumin (HSA) is a high-abundance protein in plasma that is mainly responsible for binding and transporting various endogenous or exogenous substances (*e.g.*, fatty acids, cholesterol, and many drugs); thus, it has a profound impact on the pharmacokinetic properties and efficacy of many drugs[5,6]. In patients with diabetes, HSA has a higher probability of glycation than other proteins, so it is regarded as an indicator of glycemic control[7]. Elevated glycation levels can lead to changes in the structure and function of HSA, thus influencing the normal physiological activities of the body[8]. The distinct distributions of multiple glycation sites on the three-dimensional structure of HSA cause different degrees of glycation under a range of glucose concentrations. A non-enzymatic glycation modification at the main drug-binding site substantially affects the ability of this region to bind drugs, thereby influencing the pharmacokinetic properties and efficacies of therapeutic drugs[9]. In this paper, seven aspects of HSA and its non-enzymatic glycation are reviewed.

EXPLANATION OF NON-ENZYMATIC GLYCATION AND ITS REACTION MECHANISM

Non-enzymatic glycation (sometimes described simply as glycation) is an important post-translational modification that does not involve the catalytic activity of glycosyltransferase[10]. The reaction mainly begins with a nucleophilic addition reaction between the carbonyl group of reducing sugar and the amino group of lysine, arginine, or the N-terminus of protein[11]. Fructose and lactose are important reducing sugars in food, while glucose is the main source of energy in the human body[12]. Therefore, glucose is the primary raw material for non-enzymatic glycation in the human body. The non-enzymatic glycation process is mainly divided into three steps: (1) The carbonyl group of a reducing sugar undergoes a condensation reaction with the amino group of the protein to form a thermodynamically unstable Schiff base; (2) The unstable Schiff base is converted into a relatively stable Amadori product[13,14]; and (3) Amadori product undergoes a series of spontaneous reactions (*e.g.*, dehydration, oxidation, rearrangement, and isomerization) that can generate various carbonyl compounds, such as methylglyoxal, glyoxal, 3-deoxyglucosone, and dehydroascorbic acid[15]. These carbonyl compounds usually react more strongly than the original reducing sugars and can quickly react with proteins to form various irreversible heterostructures, which are regarded as advanced glycation end products (AGEs)[16].

GENERAL STRUCTURE AND FUNCTION OF HSA

HSA is a highly abundant protein in plasma; its concentration of approximately 35-50 g/L comprises approximately 60% of the total plasma protein content[17]. It is mainly

responsible for the regulation of plasma osmotic pressure[18] and pH, and binding various endogenous or exogenous substances (*e.g.*, fatty acids, cholesterol, and many drugs)[19]. Additionally, HSA serves as an antioxidant, mediates lipid metabolism, and sequesters toxins[17]. It is composed of 585 amino acids and 17 intramolecular disulfide bonds, with a molecular weight of 66437 kDa[8]. Crystal structure analysis has shown that HSA possesses a spherical "heart-shaped" structure comprising approximately 67% of α-helices, 23% of extended chains, and 10% of β-sheets. HSA contains three homology domains: I (amino acids 1-195), II (amino acids 196-383), and III (amino acids 384-585); each of these domains contains two subdomains (A and B). The A subdomains of both domains II and III constitute the major drug-binding regions of HSA; these are regarded as sites I (amino acids 196-292) and II (amino acids 384-489)[20].

OVERVIEW OF HSA GLYCATION

Due to the high abundance of HSA, its non-enzymatic glycation represents approximately 80% of all glycation involving circulating proteins[21]. Amadori products are the main form of glycated HSA present in the body; their amounts increase as the blood glucose concentration increases in the blood of patients with diabetes[22]. The proportion of glycated HSA in healthy people is approximately 1%-10% and can increase by 2-3-fold in patients with diabetes[8,17]. Basic amino acids on HSA, specifically, 59 lysines and 24 arginines, are regarded as potential sites of glycation.

Glucose-induced modifications strongly influence HSA functional properties and have important implications for protein activity, folding, degradation, and cell function[23,24]. Although initially harmless, these modifications can become destructive and pathogenic when they become sufficiently widespread. Figure 1 shows the mechanism of the different effects of HSA glycation on the body. First, HSA glycation change the intrinsic conformations and binding efficiencies of its major binding regions, thereby changing the drug efficacy[25]. Second, the interactions of AGEs with their receptors [receptor for AGEs (RAGE)] or other macromolecules will activate various signaling pathways such as nuclear factor κB, as well as tissue damage and metabolic complications[26]. Third, glycated HSA can also stimulate platelet activation and aggregation, thereby enhancing thrombosis and inhibiting cellular uptake of glucose[27-31]. As the main drug-binding protein in plasma, HSA strongly influences drug absorption, distribution, excretion, and efficacy characteristics[32]. Changes in HSA function caused by the pathological environment can lead to unexpected types of toxicity. Drug molecules either combine with proteins and lipids in plasma or exist in a free (*i.e.*, unbound) state in the aqueous blood environment[33]. Only free drug molecules interact with their intended targets to produce therapeutic effects[33]. In some instances, the excessive modification of HSA by non-enzymatic glycation can increase the free drug concentration, which can produce severe drug toxicity[34,35].

METHODS FOR ASSESSMENT OF GLYCATED HSA

Glycated HSA has been used as a complementary indicator to standard assays involving glycated hemoglobin (HbA1c) or real-time glucose monitoring to assess glycemic control in patients with diabetes[10]. Notably, real-time glucose monitoring only provides a single data point concerning the glycemic status of patients with diabetes, while HbA1c provides an assessment of glycemic control over 2-3 mo and may be influenced by chronic kidney disease in some patients[36,37]. In contrast, glycated HSA provides an assessment of glycemic control over 21 d and can be used as an indicator with intermediate duration (*i.e.*, between real-time glucose monitoring and assessment of HbA1c)[38]. Many methods have been developed to detect and quantify glycated HSA with the aim of predicting or preventing potential complications; these methods mainly involve the determination of total glycated HSA, as well as the qualitative and quantitative assessment of HSA glycation sites.

Methods for assessment of total glycated HSA

Immunoassays such as enzyme-linked immunosorbent assays and radioimmunoassays are often used to detect total glycated HSA[39,40]. In addition, other traditional methods for evaluation of glycated HSA include boronate affinity

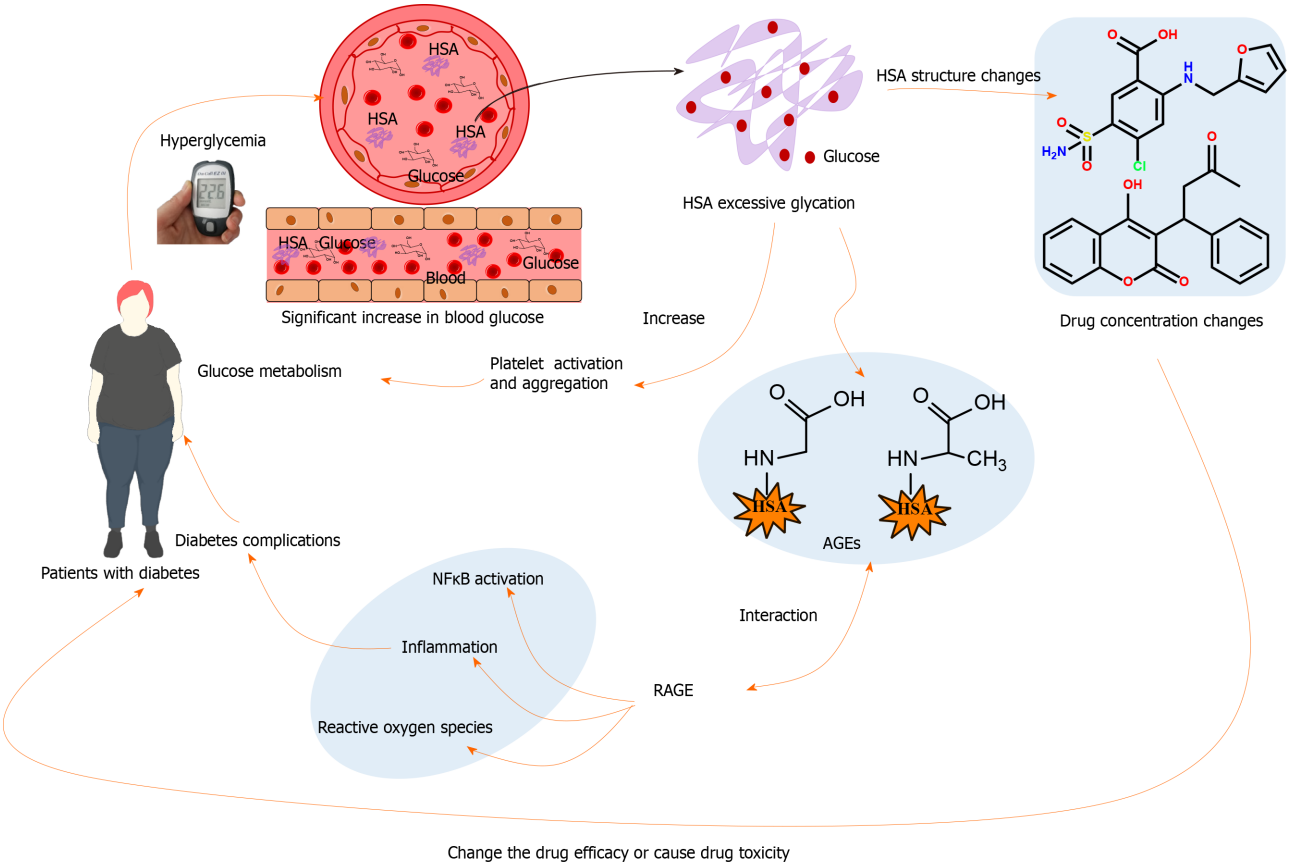

Figure 1 Mechanism of different effects of human serum albumin glycation on the human body. AGEs: Advanced glycation end products; RAGE: Receptor for advanced glycation end products; HSA: Human serum albumin.

technology; thiobarbituric acid analysis; nitro-blue tetrazolium colorimetric analysis; phenylhydrazone formation reaction; fructosamine assays; ketoamine oxidase analysis; high-performance liquid chromatography (HPLC) analysis of furosine hydrolysis by strong acid; phenylborate-containing acrylamide gel electrophoresis; and the analysis of reductive activity following alkaline solution treatment, using redox indicators[41-48]. However, the above traditional methods have their own characteristics or drawbacks. For example, colorimetric analysis methods such as nitro-blue tetrazolium and thiobarbituric acid have high unspecificity[49]; fructosamine assays provide higher specificity and reliability[50]; HPLC method has a high sensitivity[41]; phenylborate-containing acrylamide gel electrophoresis method is time-consuming and not suitable for clinical measurement[51]. In recent years, electrochemical quantitative analysis methods with high sensitivity and specificity have also been developed[52]. Intact protein analyses by high resolution mass spectrometry (MS) can also be used to determine the total glycation degree of HSA[53].

Methods for qualitative and quantitative analysis of glycation sites on HSA

HSA is rich in basic amino acids that can undergo glycation; thus, the analysis of glycation sites on HSA mainly involves the application of high-resolution MS[54]. A "Top-Down" approach combined with tandem MS is considered a standard method to accurately assess glycation sites[55-57]. In the "Top-Down" approach, HSA is first enriched and then digested with trypsin or Lys-C[7,10]. Because of glucose steric hindrance, peptides will have missed cleavage to form peptides containing glucose modifications[58]. Thus, glycated peptides exhibit a mass shift of 162 kDa in primary MS analysis, as well as a neutral loss in tandem MS analysis, and these findings can be used to locate the accurate glycation site[12]. Many types of MS with ionization modes of matrix assisted laser desorption ionization (MALDI) or electrospray ionization (*e.g.*, IT-TOF, LTQ-Orbitrap, Q-TOF, hybrid linear ion trap-Orbitrap, and MALDI-TOF MS) have been used to identify glycation sites[10,12,55,59,60]. For the quantitative analysis of glycated peptides, many approaches have been developed thus far[12,53,55,61]. Frolov *et al*[55] used the integral peak area to compare amounts of glycated peptides. In another study, isotopic labeling with ^{13}C was performed to label native proteins,

which were then digested with trypsin; the coupled ^{12}C and ^{13}C isotope peaks provided different types of quantitative information concerning the same glycated peptides[12]. Furthermore, ^{18}O- and ^{16}O-labeled H_2O has been used to hydrolyze normal and glycated HSA, respectively. The $^{16}O/^{18}O$ ratios in each digested peptide were measured to compare glycation levels[61]. Furthermore, Qiu *et al*[53] have developed an isobaric tags for relative or absolute quantitation (iTRAQ) labeling technology combined with three-stage MS (MS^3) method to compare glycation levels between healthy individuals and patients with diabetes. The iTRAQ-MS^3 method makes good use of the neutral loss of glycated peptides under collision-induced dissociation in MS/MS, and high-energy collisional dissociation in MS^3 fragmentation of the neutral loss ions were performed to precise quantification of the glycated peptides[53]. Table 1 shows the glycation sites that have been identified through qualitative and quantitative analyses. Notably, specific basic residues in HSA are involved in glycation *in vivo*[62]. Sites K525, K199, and K351 were reportedly the predominant glycation sites on HSA[62,63]. Figure 2 shows the number of reports for each potential glycation sites. Sites K12, K64, K137, K199, K233, K262, K274, K317, K378, K414, K525, K545, and K574 have been more easily identified than other sites (reported ≥ 8 times), which suggests that they are more sensitive to changes in serum glucose concentrations[7]. The underlying mechanism may be that these sites are both distributed on the HSA surface and spatially located near basic amino acids[53]. Although K199 is not completely distributed on the HSA surface, its low pKa value and spatial proximity to basic amino acids make it suitable for glycation reactions[62]. In Figure 2, we can find that some sites (*e.g.*, K20, K41, R145, R197, R209, K212, R222, R337, and K524) had never been identified in analyses of glycation modifications, indicating that they are insensitive to changes in glucose concentrations, and further explorations of the underlying mechanism are needed to determine their roles[64-71].

EFFECTS OF GLYCATION ON THE STRUCTURE AND FUNCTION OF HSA

Many functions of HSA can be attributed to its structural characteristics. The relative structural stability of HSA is mainly dependent on 17 intramolecular disulfide bonds [50]. This structural flexibility enables HSA to bind to many molecules with distinct structures[72]. The affinities of various metabolites and drugs depend on the multistage structures of binding sites, which are distributed throughout the whole HSA molecule. The major drug-binding sites of HSA are known as sites I and II[20,35,73]. Glycation contributes to various changes in HSA structure and function[74]. First, it enhances the molecular weight of HSA by attaching one or several glucose units to the basic amino acid residues of the protein. Second, glycation will change the original conformation of HSA. The intrinsic fluorescence of HSA is mainly derived from tryptophan-214 located in site I; its fluorescence is extremely sensitive to changes in the HSA environment[24,35,73]. Glycated sites located in or near Site I, such as K199, will alter the HSA structural microenvironment, thereby altering the intrinsic fluorescent characteristics of the protein. The relative fluorescence intensity of glycated HSA is reportedly reduced by 51% compared with normal HSA[75]. In addition to fluorescence chromatography, circular dichroism has also been used to study the effects of glycation on the structure of HSA[76]. Nakajou *et al*[75] used circular dichroism to compare different HSA molecules, which revealed that the secondary structure of HSA was altered after glycation with 50 mmol/L glucose. Third, the glycation of HSA will act as an oxidant and a pro-inflammatory mediator through different mechanisms[77].

Glycation-related changes in the structure of HSA can have varying effects on its abilities to bind a range of ligands. The main mechanisms that affect binding may involve steric hindrance of covalently bound glucose, the blockage of charged residues, or a combination of these two mechanisms[75]. Techniques used to study the binding affinity of glycated HSA include fluorescence spectroscopy, circular dichroism, HPLC with ultraviolet detection, and nuclear magnetic resonance[78-80]. Changes in the binding affinities of glycated HSA to various ligands are influenced by drug concentration and the degree of protein glycation[35,53,75] (see Table 2 [81-85]). Warfarin, tryptophan, and dansylsarcosine have often been used as probe compounds for HSA sites I and II in binding studies[75,76]. *In vitro* analysis has shown that HSA glycation with a range of glucose concentrations (2.5 mmol/L, 12.5 mmol/L, and 50 mmol/L) enhanced the binding of warfarin, but weakened the binding of dansylsarcosine[75]. Another study showed that both *ex vivo* (purified from the plasma of patients with diabetes) and *in vitro* glycated HSA exhibited weakened binding

Table 1 Review of the *in vivo* glycation sites of human serum albumin

Ref.	Glycation sites reported so far	Analysis tools
Iberg *et al*[63]	HSA from a diabetic patient: Lys-12, Lys-199, Lys-233, Lys-281, Lys-317, Lys-351, Lys-439, Lys-525, Lys-534	Amino acid analysis after hydrolysis in HCl
Garlick *et al*[64]	Freshly purified human serum albumin: Lys-525	Cation exchange chromatography
Frolov *et al*[55]	HSA from five T2DM patients: Lys-12, Lys-51, Lys-64, Lys-162, Lys-174, Lys-181, Lys-233, Lys-262, Lys-276, Lys-351, Lys-359, Lys-378, Lys-414, Lys-475, Lys-525, Lys-545	Q-TOF-MS
Kisugi *et al*[56]	HSA from a female diabetic patients: Lys-64/Lys-73, Lys-199, Lys-136/ Lys-137, Lys-233, Lys-274/Lys-276, Lys-317, Lys-389, Lys-439, Lys-534, Lys-525	QSTAR Pulsar-i mass spectrometer
Frolov *et al*[57]	HSA from 5 T2DM patients and 4 healthy subjects: Lys-12, Lys-51[1], Lys-64[1], Lys-73, Lys-93, Lys-137, Lys-162, Lys-174[1], Lys-181[1], Lys-205, Lys-233[1], Lys-262[1], Lys-274, Lys-351, Lys-359[1], Lys-378[1], Lys-414, Lys-475, Lys-525, Lys-545[1], Lys-557[1] (detected only in diabetic samples), Lys-574	Nano-ESI-LTQ Orbitrap XL MS with ETD
Bai *et al*[10]	HSA from a healthy subject and a diabetic patient: Lys-64, Lys-93, Lys-190, Lys-199, Lys-205, Lys-225, Lys-233, Lys-240, Lys-262, Lys-274, Lys-281, Lys-317, Lys-323, Lys-351, Lys-372, Lys-378, Lys-413, Lys-432, Lys-475, Lys-525, Lys-545, Lys-557, Lys-557/ Lys-560/ Lys-564, Lys-564, Lys-573/ Lys-574	IT-TOF-MS/MS
Zhang *et al*[7]	HSA from clinical T2DM, IGT, NGT and 389 volunteers: Lys-12/ Lys-20[1], Arg-144, Arg-186/ Lys-190[1], Arg-222/ Lys-225, Lys-240, Arg-336, Lys-372, Lys-414/ Arg-428[1]. (8 glucose sensitive sites)	Agilent MSD trap
Anguizola *et al*[59]	HSA from individual clinical plasma samples: Arg-10, Lys-12, Arg-10/Lys-12[1], Arg-98[1], Arg-160, Lys-162, Lys-190, Lys-199, Lys-276, Lys-281, Lys-276/Lys-281[1], Lys-286[1], Lys-313, Lys-317, Lys-372, Lys-428, Lys-432, Arg-484, Arg-485, Arg-484/ Arg-485[1], Lys-545, Lys-557, Lys-560, Lys-564[1], Lys-573/ Lys-574[1]	MALDI-TOF-MS
Priego-Capote *et al*[12]	HSA from human Plasma: Lys-64, Lys-73, Lys-93, Lys-106, Lys-136, Lys-137, Lys-159, Lys-174, Lys-181, Lys-195, Arg-218, Lys-233, Lys -240, Lys-262, Lys-274, Lys-323, Lys-359, Lys-372, Lys-378, Lys-389, Lys -402, Lys-413, Lys-432, Lys-436, Lys-439, Lys-444, Lys-466, Arg-472, Lys-475, Lys-500, Lys-519, Lys -525, Lys-573	Hybrid linear ion trap-Orbitrap MS
Korwar *et al*[65]	HSA from clinical plasma samples: Lys-12, Lys -64[1], Lys -136, Lys-137, Lys-159[1], Lys-402[1], Lys-414[1], Lys-466[1], Lys-525[1]	Hybrid quadruple Q-Exactive Orbitrap MS
Zhang *et al*[60]	HSA from 12 NGT, 11 IGT and 8 T2DM: Lys-4[1], Lys-12, Lys-51, Lys-64[1], Lys-73, Lys-136, Lys-137, Lys-159, Lys-162, Lys-181[1], Lys-190[1], Lys-195, Lys-199[1], Lys-205, Lys-225, Lys-233[1], Lys-262, Lys-274, Lys-276, Lys-317[1], Lys-351, Lys-378, Lys-414, Lys-432[1], Lys-436[1], Lys-475, Lys-525, Lys-538, Lys-545, Lys-562[1], Lys-573, Lys-574	Ion Trap LC-MS
Miyamoto *et al*[66]	HSA from 8 diabetic patients: Lys-51, Lys-64/ Lys-73, Lys-136/ Lys-137, Lys-159/ Lys-162, Lys-190/ Lys-195/ Lys-199/ Lys-205, Lys-233, Lys-262, Lys-274/ Lys-276, Lys-313/ Lys-317, Lys-351, Lys-378/ Lys-389, Lys-432/ Lys-436/ Lys-439, Lys-525, Lys-534/ Lys -536/ Lys-538/ Lys-541, Lys -545, Lys-573/ Lys-574	QSTAR Pulsar-i MS
Brede *et al*[67]	HSA from plasma: Lys-12, Lys-137, Lys-414, Lys-525[1]	Q-TOF MS
Spiller *et al*[68]	HSA from 48 T2DM patients and 48 non-diabetic: Lys-64, Lys-73, Lys-93, Lys-174, Lys-181, Lys-233, Lys-262, Lys-359, Lys-378, Lys-414, Lys-525, Lys-545, Lys-574	QTRAP 4000
Spiller *et al*[69]	HSA from 5 T2DM patients and 5 non-diabetic individuals: Lys-64[1], Lys-73[1], Lys-181[1], Lys-262[1], Lys-378[1], Lys-574[1]	ESI-QqLIT-MS (4000
Takátsy *et al*[70]	HSA from diabetic patients and healthy individuals: Arg-81, Lys-93, Arg-98, Lys-106, Arg-114, Lys-190, Lys-199, Arg-218, Arg-257, Lys-276, Lys-317, Arg -348, Lys-372, Lys-378, Lys-389, Lys-413, Lys-436, Lys-439, Lys-444, Lys-466, Arg-484, Arg-485, Lys-500, Lys-519, Arg-521, Lys-564, Lys-536, Lys-538, Arg-445, Lys-541, Lys-560, Lys-573	MALDI TOF MS
Greifenhagen *et al*[71]	HSA from 5 diabetic patients: Lys-12, Lys-64, Lys-137, Lys-190, Lys-199, Lys-274, Lys-276, Lys-525	ESI-Orbitrap-MS
Qiu *et al*[53]	HSA from 4 diabetic patients and 4 healthy subjects: Lys-4, Lys-12, Lys-51[1], Lys-64[1], Lys-73, Arg-81, Lys-93[1], Arg-98, Arg-117, Lys-136, Lys-137, Lys-162[1], Lys-174, Lys-181, Arg-186, Lys-199[1], Lys-205, Lys-233[1], Lys-240, Arg-257, Lys-262[1], Lys-274, Lys-276, Lys-281, Lys-286, Lys-313[1], Lys-317, Lys-323[1], Lys-351, Lys-359, Lys-372, Lys -378[1], Lys-389, Lys-402[1], Lys-410, Lys-414[1], Lys-436, Lys-439, Lys-466[1], Lys-475[1], Lys-519, Lys-525[1], Lys-538, Lys-541, Lys-545[1], Lys-557[1], Lys-564[1], Lys-573, Lys-574[1]	LTQ Orbitrap Velos Pro MS

[1]Represents glycation sites detected at higher quantities in diabetic patients than in healthy individuals. HSA: Human serum albumin; ESI: Electrospray ionization; NGT: Normal glucose tolerance; T2DM: Type 2 diabetes mellitus; MS: Mass spectrometry.

interactions with warfarin[35]. Joseph *et al*[76] proved that the binding of L-tryptophan was enhanced by 4.7-5.8 fold under glycation conditions similar to those in patients with diabetes, although the binding of warfarin remained unchanged. Notably, the above contradictory results concerning warfarin were obtained under relatively nonphysiological conditions *in vitro*. Qiu *et al*[53] found that the affinity of warfarin for HSA was greater in plasma from patients with diabetes. The level of free warfarin was also reduced in subsequent pharmacokinetic experiments[53]. Furthermore, a retrospective clinical study revealed that the anticoagulant effect of warfarin was

Table 2 Effects of glycation on the binding of human serum albumin to various ligands

Ref.	Ligands	*In vivo/ vitro/ex vivo*	Glycation level of HSA	Binding affinity
Nakajou *et al*[75]	Warfarin	*In vitro*	HSA glycated with 2.5 mmol/L, 12.5 mmol/L, and 50 mmol/L glucose	↑
Baraka-Vidot *et al*[35]	Warfarin	*In vitro* and *Ex vivo*	HSA purified from blood and HSA glycated with 25 mmol/L or 100 mmol/L glucose	↓
Joseph *et al*[76]	Warfarin	*In vitro*	HSA glycated with 0.5 mol/L glucose	→
Qiu *et al*[53]	Warfarin	*In vivo*	HSA from diabetic patients	↑
Joseph *et al*[76]	Tryptophan	*In vitro*	HSA glycated with 0.5 mol/L glucose	↑4.7-5.8-fold
Nakajou *et al*[75]	Dansylsarcosine	*In vitro*	HSA glycated with 2.5 mmol/L, 12.5 mmol/L, and 50 mmol/L glucose	↓
Qiu *et al*[53]	Heparin	*In vitro* and *in vivo*	HSA from diabetic patients	→
Guerin-Dubourg *et al*[81]	Copper	*In vivo*	HSA purified from diabetic patients and control individuals	↓16%
Koizumi *et al*[82]	Furosemide	*In vitro*	Prepared from HSA, and commercial HSA	↓
Okabe *et al*[83]	Phenylbutazone	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓
Yamazaki *et al*[84]	Fatty acids	*In vitro*	HSA glycated with 100 mmol/L glucose	↓
Karp *et al*[85]	Diazepam	*In vitro*	HSA glycated with 140 mmol/L glucose	→
Karp *et al*[85]	Bilirubin	*In vitro*	HSA glycated with 140 mmol/L glucose	↓30%
Okabe *et al*[83]	Ibuprofen	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓20
Okabe *et al*[83]	Dansylproline	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓25%
Okabe *et al*[83]	Flufenamic acid	*In vitro*	Each mole of HSA contains 1.94 moles of glucose	↓
Koizumi *et al*[82]	Naproxen	*In vitro*	Prepared from HSA, and commercial HSA	→

"→": No change; "↑": Increase; "↓": Decrease; HSA: Human serum albumin.

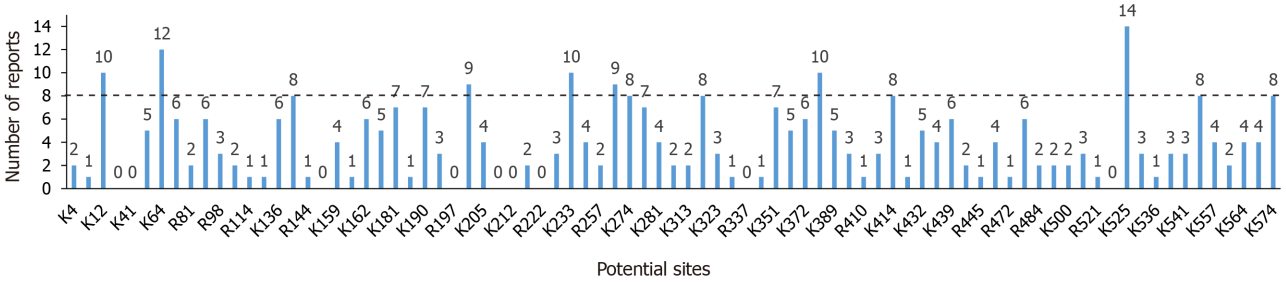

Figure 2 Number of reports for each potential glycation site. Dotted line represents that the number of reports reaches 8 times.

reduced in patients with diabetes[53]. These *in vivo* findings may provide better reference data with respect to warfarin binding.

HSA GLYCATION AND COMPLICATIONS

Chronic hyperglycemia is the primary condition associated with complications of diabetes. Hyperglycemia leads to excessive irreversible accumulation of AGEs on long-lived proteins, such as HSA and HbA1c. The degrees and durations of protein exposure to abnormally high levels of glucose are closely related to the degrees and rates of progression of nephropathy, stroke, neuropathy, retinopathy, and cardiovascular disease[86]. There remain questions concerning how the accumulation of AGEs promotes the development of these lesions. There are three main consequences of the formation of AGEs: (1) Cross-linking of various extracellular proteins[87]; (2) Changes in cell–matrix interactions[88,89]; and (3) Changes in DNA

structure and function[90]. HSA is the main protein in blood circulation; patients with diabetes exhibit significantly greater levels of the HSA-related AGEs[91]. Interactions between AGEs and RAGEs alter cellular signals and gene expression, thereby enhancing the secretion of pro-inflammatory molecules and leading to oxidative stress reactions in patients with diabetes[92].

HSA GLYCATION AND CLINICAL APPLICATIONS

Glycation is a continuous process in the human body. Elevated levels of glycated proteins are associated with elevated levels of blood glucose in patients with diabetes. Thus, there is considerable interest in measuring the glycation levels in patients with diabetes; these data can be used for diagnosis, treatment, and prognosis[93,94]. For many years, HbA1c has been used for the clinical monitoring of long-term blood glucose control[95]. However, HbA1c monitoring has some limitations. Because the lifespan of HbA1c is approximately 3 mo, rapid changes in serum glucose status (*e.g.*, treatment response) are not clearly reflected in HbA1c measurements[96,97]. In some individuals, an abnormally elevated HbA1c value may be recorded, such as patients with hemoglobin variants[96,98], patients with rapid changes in glucose control, patients with iron-deficiency anemia, patients with HIV, or pregnant patients[99-102]. In patients with reduced erythrocyte lifespan, such as those with liver cirrhosis[103], hemolytic anemia[104], chronic kidney disease, and/or hemorrhage, the recorded values of HbA1c will decrease[105,106]. HSA glycation has been suggested as an alternative clinical indicator to circumvent many limitations of HbA1c assessment. The level of HSA glycation is not affected by hemoglobin genetic variations or changes in erythrocyte lifespan[107]. Compared with HbA1c, glycated HSA has a much shorter half-life and is therefore more sensitive to changes in glycemic status. The levels of glycated HSA reflect the average plasma glucose level over a 2-wk interval[94,108]. Therefore, glycated HSA is a more dynamic indicator of glycemic control, which can be used to evaluate the drug treatment efficacy and short-term changes in glucose control. In patients with pre-diabetes, the total degree of HSA glycation does not provide all possible information regarding short-term fluctuations in plasma glucose concentrations because of the high number of possible glycation sites. Therefore, the comparison of the glycation degree of specific HSA sites sensitive to glucose (*e.g.*, K525 and K199) can be used as clinical biomarkers for the occurrence and early diagnosis of diabetes[53,65]. However, it is noteworthy that glycated HSA levels are also influenced by hypoalbuminemic conditions such as malnutrition, nephrotic syndrome, liver cirrhosis, or other liver and renal disease[109]. Further verification is needed to determine whether and how glycated albumin can be used as an indicator of hyperglycemia under these conditions.

CONCLUSION

Hyperglycemia leads to enhanced HSA glycation in patients with diabetes; this highly non-enzymatic glycation at multiple sites can impact the function of HSA as a drug carrier. In this review, we have presented a detailed summary of non-enzymatic glycation sites identified thus far *in vivo*; we have also discussed the impacts of non-enzymatic glycation on the three-dimensional structure and biological functions of HSA. It would be useful to determine how modifications in HSA glycation affect drug treatments for a range of diseases. Glycated HSA may serve as a new clinical indicator for assessment of glycemic control, potentially as an alternative for the long-term indicator HbA1c. Additional *in vivo* studies are needed to determine the effects of glycated HSA on combinations and efficacies of various drugs, thereby providing reference data to aid in the guidance of clinical treatment for patients with diabetes.

REFERENCES

1 **Huang Y**. IDF Diabetes Atlas 8th Edition. International diabetes federation 2017 [cited 20 April 2021]. Available from: https://diabetestalk.net/diabetes/idf-diabetes-atlas-8th-edition

2 **Yamagishi S**, Maeda S, Matsui T, Ueda S, Fukami K, Okuda S. Role of advanced glycation end products (AGEs) and oxidative stress in vascular complications in diabetes. *Biochim Biophys Acta* 2012; **1820**: 663-671 [PMID: 21440603 DOI: 10.1016/j.bbagen.2011.03.014]

3 **Daneman D**. Diabetes-related mortality. A pediatrician's view. *Diabetes Care* 2001; **24**: 801-802 [PMID: 11347732 DOI: 10.2337/diacare.24.5.801]

4 **Chilelli NC**, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: a "glycoxidation-centric" point of view. *Nutr Metab Cardiovasc Dis* 2013; **23**: 913-919 [PMID: 23786818 DOI: 10.1016/j.numecd.2013.04.004]

5 **Ghuman J**, Zunszain PA, Petitpas I, Bhattacharya AA, Otagiri M, Curry S. Structural basis of the drug-binding specificity of human serum albumin. *J Mol Biol* 2005; **353**: 38-52 [PMID: 16169013 DOI: 10.1016/j.jmb.2005.07.075]

6 **Bhat S**, Jagadeeshaprasad MG, Venkatasubramani V, Kulkarni MJ. Abundance matters: role of albumin in diabetes, a proteomics perspective. *Expert Rev Proteomics* 2017; **14**: 677-689 [PMID: 28689445 DOI: 10.1080/14789450.2017.1352473]

7 **Zhang M**, Xu W, Deng Y. A new strategy for early diagnosis of type 2 diabetes by standard-free, label-free LC-MS/MS quantification of glycated peptides. *Diabetes* 2013; **62**: 3936-3942 [PMID: 23894188 DOI: 10.2337/db13-0347]

8 **Shaklai N**, Garlick RL, Bunn HF. Nonenzymatic glycosylation of human serum albumin alters its conformation and function. *J Biol Chem* 1984; **259**: 3812-3817 [PMID: 6706980]

9 **Szkudlarek A**, Wilk M, Maciążek-Jurczyk M. In Vitro Investigations of Acetohexamide Binding to Glycated Serum Albumin in the Presence of Fatty Acid. *Molecules* 2020; **25**: 2340 [PMID: 32429512 DOI: 10.3390/molecules25102340]

10 **Bai X**, Wang Z, Huang C, Chi L. Investigation of non-enzymatic glycosylation of human serum albumin using ion trap-time of flight mass spectrometry. *Molecules* 2012; **17**: 8782-8794 [PMID: 22832880 DOI: 10.3390/molecules17088782]

11 **Anguizola J**, Matsuda R, Barnaby OS, Hoy KS, Wa C, DeBolt E, Koke M, Hage DS. Review: Glycation of human serum albumin. *Clin Chim Acta* 2013; **425**: 64-76 [PMID: 23891854 DOI: 10.1016/j.cca.2013.07.013]

12 **Priego-Capote F**, Scherl A, Müller M, Waridel P, Lisacek F, Sanchez JC. Glycation isotopic labeling with 13C-reducing sugars for quantitative analysis of glycated proteins in human plasma. *Mol Cell Proteomics* 2010; **9**: 579-592 [PMID: 19955080 DOI: 10.1074/mcp.M900439-MCP200]

13 **Reynolds TM**. Chemistry of nonenzymic browning. I. The reaction between aldoses and amines. *Adv Food Res* 1963; **12**: 1-52 [PMID: 14280861 DOI: 10.1016/s0065-2628(08)60005-1]

14 **Reynolds TM**. Chemistry of nonenzymic browning. II. *Adv Food Res* 1965; **14**: 167-283 [PMID: 5341910 DOI: 10.1016/s0065-2628(08)60149-4]

15 **Thornalley PJ**, Langborg A, Minhas HS. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem J* 1999; **344** Pt 1: 109-116 [PMID: 10548540]

16 **Brownlee M**, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 1988; **318**: 1315-1321 [PMID: 3283558 DOI: 10.1056/NEJM198805193182007]

17 **Bourdon E**, Loreau N, Blache D. Glucose and free radicals impair the antioxidant properties of serum albumin. *FASEB J* 1999; **13**: 233-244 [PMID: 9973311 DOI: 10.1096/fasebj.13.2.233]

18 **Scatchard G**, Batchelder AC, Brown A. Chemical, clinical, and immunological studies on the products of human plasma fractionation. vi. The osmotic pressure of plasma and of serum albumin. *J Clin Invest* 1944; **23**: 458-464 [PMID: 16695124 DOI: 10.1172/JCI101513]

19 **Anguizola JA**, Basiaga SB, Hage DS. Effects of Fatty Acids and Glycation on Drug Interactions with Human Serum Albumin. *Curr Metabolomics* 2013; **1**: 239-250 [PMID: 24349966 DOI: 10.2174/2213235x1130100005]

20 **Perters T**. All about albumin: Biochemistry, Genetics and Medical Application. Academic Press: San Diego, 1995

21 **Schleicher ED**, Mayer R, Wagner EM, Gerbitz KD. Is serum fructosamine assay specific for determination of glycated serum protein? *Clin Chem* 1988; **34**: 320-323 [PMID: 3124979]

22 **Cohen MP**. Intervention strategies to prevent pathogenetic effects of glycated albumin. *Arch Biochem Biophys* 2003; **419**: 25-30 [PMID: 14568005 DOI: 10.1016/j.abb.2003.08.012]

23 **Voziyan PA**, Khalifah RG, Thibaudeau C, Yildiz A, Jacob J, Serianni AS, Hudson BG. Modification of proteins *in vitro* by physiological levels of glucose: pyridoxamine inhibits conversion of Amadori intermediate to advanced glycation end-products through binding of redox metal ions. *J Biol Chem* 2003; **278**: 46616-46624 [PMID: 12975371 DOI: 10.1074/jbc.M307155200]

24 **Mendez DL**, Jensen RA, McElroy LA, Pena JM, Esquerra RM. The effect of non-enzymatic glycation on the unfolding of human serum albumin. *Arch Biochem Biophys* 2005; **444**: 92-99 [PMID: 16309624 DOI: 10.1016/j.abb.2005.10.019]

25 **Oettl K**, Stauber RE. Physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties. *Br J Pharmacol* 2007; **151**: 580-590 [PMID: 17471184 DOI: 10.1038/sj.bjp.0707251]

26 **Cohen MP**, Shea E, Chen S, Shearman CW. Glycated albumin increases oxidative stress, activates NF-kappa B and extracellular signal-regulated kinase (ERK), and stimulates ERK-dependent transforming growth factor-beta 1 production in macrophage RAW cells. *J Lab Clin Med* 2003; **141**: 242-249 [PMID: 12677169 DOI: 10.1067/mLc.2003.27]

27 **Miele C**, Riboulet A, Maitan MA, Oriente F, Romano C, Formisano P, Giudicelli J, Beguinot F, Van Obberghen E. Human glycated albumin affects glucose metabolism in L6 skeletal muscle cells by

impairing insulin-induced insulin receptor substrate (IRS) signaling through a protein kinase C alpha-mediated mechanism. *J Biol Chem* 2003; **278**: 47376-47387 [PMID: 12970360 DOI: 10.1074/jbc.M301088200]

28 **Unoki H**, Bujo H, Yamagishi S, Takeuchi M, Imaizumi T, Saito Y. Advanced glycation end products attenuate cellular insulin sensitivity by increasing the generation of intracellular reactive oxygen species in adipocytes. *Diabetes Res Clin Pract* 2007; **76**: 236-244 [PMID: 17097186 DOI: 10.1016/j.diabres.2006.09.016]

29 **Unoki H**, Yamagishi S. Advanced glycation end products and insulin resistance. *Curr Pharm Des* 2008; **14**: 987-989 [PMID: 18473850 DOI: 10.2174/138161208784139747]

30 **Rubenstein DA**, Yin W. Glycated albumin modulates platelet susceptibility to flow induced activation and aggregation. *Platelets* 2009; **20**: 206-215 [PMID: 19437339 DOI: 10.1080/09537100902795492]

31 **Doweiko JP**, Bistrian BR. The effect of glycosylated albumin on platelet aggregation. *JPEN J Parenter Enteral Nutr* 1994; **18**: 516-520 [PMID: 7602726 DOI: 10.1177/0148607194018006516]

32 **Wang Y**, Wang S, Huang M. Structure and enzymatic activities of human serum albumin. *Curr Pharm Des* 2015; **21**: 1831-1836 [PMID: 25732556 DOI: 10.2174/1381612821666150302113906]

33 **Smith DA**, Di L, Kerns EH. The effect of plasma protein binding on *in vivo* efficacy: misconceptions in drug discovery. *Nat Rev Drug Discov* 2010; **9**: 929-939 [PMID: 21119731 DOI: 10.1038/nrd3287]

34 **Dasgupta A**. Clinical utility of free drug monitoring. *Clin Chem Lab Med* 2002; **40**: 986-993 [PMID: 12476936 DOI: 10.1515/CCLM.2002.172]

35 **Baraka-Vidot J**, Guerin-Dubourg A, Bourdon E, Rondeau P. Impaired drug-binding capacities of in vitro and in vivo glycated albumin. *Biochimie* 2012; **94**: 1960-1967 [PMID: 22627382 DOI: 10.1016/j.biochi.2012.05.017]

36 **Vos FE**, Schollum JB, Walker RJ. Glycated albumin is the preferred marker for assessing glycaemic control in advanced chronic kidney disease. *NDT Plus* 2011; **4**: 368-375 [PMID: 25984197 DOI: 10.1093/ndtplus/sfr140]

37 **Neelofar K**, Ahmad J. Amadori albumin in diabetic nephropathy. *Indian J Endocrinol Metab* 2015; **19**: 39-46 [PMID: 25593824 DOI: 10.4103/2230-8210.146863]

38 **Raghav A**, Ahmad J. Glycated serum albumin: a potential disease marker and an intermediate index of diabetes control. *Diabetes Metab Syndr* 2014; **8**: 245-251 [PMID: 25311816 DOI: 10.1016/j.dsx.2014.09.017]

39 **Ikeda K**, Sakamoto Y, Kawasaki Y, Miyake T, Tanaka K, Urata T, Katayama Y, Ueda S, Horiuchi S. Determination of glycated albumin by enzyme-linked boronate immunoassay (ELBIA). *Clin Chem* 1998; **44**: 256-263 [PMID: 9474021]

40 **Ohe Y**, Matsuura M, Nakajima Y, Shin S, Hashimoto F, Hirota M, Shima K. Radioimmunoassay of glycosylated albumin with monoclonal antibody to glucitol-lysine. *Clin Chim Acta* 1987; **169**: 229-238 [PMID: 3123099 DOI: 10.1016/0009-8981(87)90323-8]

41 **Shima K**, Ito N, Abe F, Hirota M, Yano M, Yamamoto Y, Uchida T, Noguchi K. High-performance liquid chromatographic assay of serum glycated albumin. *Diabetologia* 1988; **31**: 627-631 [PMID: 3220198 DOI: 10.1007/BF00264772]

42 **Dolhofer R**, Wieland OH. Improvement of the thiobarbituric acid assay for serum glycosylprotein determination. *Clin Chim Acta* 1981; **112**: 197-204 [PMID: 7237826 DOI: 10.1016/0009-8981(81)90378-8]

43 **Elder E**, Kennedy L. Rapid, accurate colorimetric assay of non-enzymatically glycosylated serum proteins. *Diabetologia* 1983; **24**: 70-71 [PMID: 6825979 DOI: 10.1007/BF00275952]

44 **Baker JR**, Metcalf PA, Johnson RN, Newman D, Rietz P. Use of protein-based standards in automated colorimetric determinations of fructosamine in serum. *Clin Chem* 1985; **31**: 1550-1554 [PMID: 4028402]

45 **Mashiba S**, Uchida K, Okuda S, Tomita S. Measurement of glycated albumin by the nitroblue tetrazolium colorimetric method. *Clin Chim Acta* 1992; **212**: 3-15 [PMID: 1486680 DOI: 10.1016/0009-8981(92)90133-b]

46 **Acharya AS**, Manning JM. Amadori rearrangement of glyceraldehyde-hemoglobin Schiff base adducts. A new procedure for the determination of ketoamine adducts in proteins. *J Biol Chem* 1980; **255**: 7218-7224 [PMID: 7391079]

47 **Ghiggeri GM**, Candiano G, Delfino G, Queirolo C, Pallavicini G, Ginevri F. Characterisation of the phenylhydrazone derivatives of "glycated albumin" purified from diabetic sera. *Carbohydr Res* 1986; **153**: 314-317 [PMID: 3779698 DOI: 10.1016/s0008-6215(00)90273-8]

48 **Kouzuma T**, Uemastu Y, Usami T, Imamura S. Study of glycated amino acid elimination reaction for an improved enzymatic glycated albumin measurement method. *Clin Chim Acta* 2004; **346**: 135-143 [PMID: 15256314 DOI: 10.1016/j.cccn.2004.02.019]

49 **Vanhaeverbeek M**, Brohee D, Lefevre A, Piro P, Kennes B, Neve P. Thiobarbiturate and fructosamine assays: significance and interest of the borohydride blank. *Acta Diabetol* 1994; **31**: 43-46 [PMID: 8043897 DOI: 10.1007/BF00580760]

50 **Rondeau P**, Bourdon E. The glycation of albumin: structural and functional impacts. *Biochimie* 2011; **93**: 645-658 [PMID: 21167901 DOI: 10.1016/j.biochi.2010.12.003]

51 **Morais MP**, Mackay JD, Bhamra SK, Buchanan JG, James TD, Fossey JS, van den Elsen JM. Analysis of protein glycation using phenylboronate acrylamide gel electrophoresis. *Proteomics* 2010; **10**: 48-58 [PMID: 19899078 DOI: 10.1002/pmic.200900269]

52 **Attar AM**, Richardson MB, Speciale G, Majumdar S, Dyer RP, Sanders EC, Penner RM, Weiss GA. Electrochemical Quantification of Glycated and Non-glycated Human Serum Albumin in Synthetic Urine. *ACS Appl Mater Interfaces* 2019; **11**: 4757-4765 [PMID: 30668098 DOI: 10.1021/acsami.8b16071]

53 **Qiu H**, Jin L, Chen J, Shi M, Shi F, Wang M, Li D, Xu X, Su X, Yin X, Li W, Zhou X, Linhardt RJ, Wang Z, Chi L, Zhang Q. Comprehensive Glycomic Analysis Reveals That Human Serum Albumin Glycation Specifically Affects the Pharmacokinetics and Efficacy of Different Anticoagulant Drugs in Diabetes. *Diabetes* 2020; **69**: 760-770 [PMID: 31974145 DOI: 10.2337/db19-0738]

54 **Niwa T**. Mass spectrometry for the study of protein glycation in disease. *Mass Spectrom Rev* 2006; **25**: 713-723 [PMID: 16526005 DOI: 10.1002/mas.20089]

55 **Frolov A**, Hoffmann R. Identification and relative quantification of specific glycation sites in human serum albumin. *Anal Bioanal Chem* 2010; **397**: 2349-2356 [PMID: 20496030 DOI: 10.1007/s00216-010-3810-9]

56 **Kisugi R**, Kouzuma T, Yamamoto T, Akizuki S, Miyamoto H, Someya Y, Yokoyama J, Abe I, Hirai N, Ohnishi A. Structural and glycation site changes of albumin in diabetic patient with very high glycated albumin. *Clin Chim Acta* 2007; **382**: 59-64 [PMID: 17482589 DOI: 10.1016/j.cca.2007.04.001]

57 **Frolov A**, Blüher M, Hoffmann R. Glycation sites of human plasma proteins are affected to different extents by hyperglycemic conditions in type 2 diabetes mellitus. *Anal Bioanal Chem* 2014; **406**: 5755-5763 [PMID: 25074545 DOI: 10.1007/s00216-014-8018-y]

58 **Lee JY**, Kim JY, Cheon MH, Park GW, Ahn YH, Moon MH, Yoo JS. MRM validation of targeted nonglycosylated peptides from N-glycoprotein biomarkers using direct trypsin digestion of undepleted human plasma. *J Proteomics* 2014; **98**: 206-217 [PMID: 24434586 DOI: 10.1016/j.jprot.2014.01.003]

59 **Anguizola J**, Joseph KS, Barnaby OS, Matsuda R, Alvarado G, Clarke W, Cerny RL, Hage DS. Development of affinity microcolumns for drug-protein binding studies in personalized medicine: interactions of sulfonylurea drugs with *in vivo* glycated human serum albumin. *Anal Chem* 2013; **85**: 4453-4460 [PMID: 23544441 DOI: 10.1021/ac303734c]

60 **Zhang Q**, Tang N, Schepmoes AA, Phillips LS, Smith RD, Metz TO. Proteomic profiling of nonenzymatically glycated proteins in human plasma and erythrocyte membranes. *J Proteome Res* 2008; **7**: 2025-2032 [PMID: 18396901 DOI: 10.1021/pr700763r]

61 **Barnaby OS**, Cerny RL, Clarke W, Hage DS. Quantitative analysis of glycation patterns in human serum albumin using 16O/18O-labeling and MALDI-TOF MS. *Clin Chim Acta* 2011; **412**: 1606-1615 [PMID: 21601565 DOI: 10.1016/j.cca.2011.05.012]

62 **Neelofar K**, Ahmad J. An overview of *in vitro* and *in vivo* glycation of albumin: a potential disease marker in diabetes mellitus. *Glycoconj J* 2017; **34**: 575-584 [PMID: 28812216 DOI: 10.1007/s10719-017-9789-0]

63 **Iberg N**, Flückiger R. Nonenzymatic glycosylation of albumin in vivo. Identification of multiple glycosylated sites. *J Biol Chem* 1986; **261**: 13542-13545 [PMID: 3759977 DOI: 10.1016/S0021-9258(18)67052-8]

64 **Garlick RL**, Mazer JS. The principal site of nonenzymatic glycosylation of human serum albumin in vivo. *J Biol Chem* 1983; **258**: 6142-6146 [PMID: 6853480]

65 **Korwar AM**, Vannuruswamy G, Jagadeeshaprasad MG, Jayaramaiah RH, Bhat S, Regin BS, Ramaswamy S, Giri AP, Mohan V, Balasubramanyam M, Kulkarni MJ. Development of Diagnostic Fragment Ion Library for Glycated Peptides of Human Serum Albumin: Targeted Quantification in Prediabetic, Diabetic, and Microalbuminuria Plasma by Parallel Reaction Monitoring, SWATH, and MSE. *Mol Cell Proteomics* 2015; **14**: 2150-2159 [PMID: 26023067 DOI: 10.1074/mcp.M115.050518]

66 **Miyamoto H**, Kohzuma T, Ohnishi A. Changes in the albumin glycation site, plasma pentosidine and esRAGE concentrations before and after intensive diabetic treatment in patients with abnormally high glycated albumin levels. *Ann Clin Biochem* 2018; **55**: 84-91 [PMID: 28504612 DOI: 10.1177/0004563216687787]

67 **Brede C**, Hop B, Jørgensen K, Skadberg Ø. Measurement of glycated albumin in serum and plasma by LC-MS/MS. *Scand J Clin Lab Invest* 2016; **76**: 195-201 [PMID: 26898156 DOI: 10.3109/00365513.2015.1129671]

68 **Spiller S**, Li Y, Blüher M, Welch L, Hoffmann R. Glycated lysine-141 in haptoglobin improves the diagnostic accuracy for type 2 diabetes mellitus in combination with glycated hemoglobin HbA_{1c} and fasting plasma glucose. *Clin Proteomics* 2017; **14**: 10 [PMID: 28360826 DOI: 10.1186/s12014-017-9145-1]

69 **Spiller S**, Frolov A, Hoffmann R. Quantification of Specific Glycation Sites in Human Serum Albumin as Prospective Type 2 Diabetes Mellitus Biomarkers. *Protein Pept Lett* 2017; **24**: 887-896 [PMID: 28155619 DOI: 10.2174/0929866524666170202124120]

70 **Takátsy A**, Böddi K, Nagy L, Nagy G, Szabó S, Markó L, Wittmann I, Ohmacht R, Ringer T, Bonn GK, Gjerde D, Szabó Z. Enrichment of Amadori products derived from the nonenzymatic glycation of proteins using microscale boronate affinity chromatography. *Anal Biochem* 2009; **393**: 8-22 [PMID: 19524544 DOI: 10.1016/j.ab.2009.06.007]

71 **Greifenhagen U**, Frolov A, Blüher M, Hoffmann R. Plasma Proteins Modified by Advanced Glycation End Products (AGEs) Reveal Site-specific Susceptibilities to Glycemic Control in Patients with Type 2 Diabetes. *J Biol Chem* 2016; **291**: 9610-9616 [PMID: 26933035 DOI: 10.1074/jbc.M115.702860]

72 **Weber G**. Energetics of ligand binding to proteins. *Adv Protein Chem* 1975; **29**: 1-83 [PMID: 1136898 DOI: 10.1016/s0065-3233(08)60410-6]

73 **Wang Y**, Yu H, Shi X, Luo Z, Lin D, Huang M. Structural mechanism of ring-opening reaction of glucose by human serum albumin. *J Biol Chem* 2013; **288**: 15980-15987 [PMID: 23592780 DOI: 10.1074/jbc.M113.467027]

74 **Cao H**, Chen T, Shi Y. Glycation of human serum albumin in diabetes: impacts on the structure and function. *Curr Med Chem* 2015; **22**: 4-13 [PMID: 25245514 DOI: 10.2174/0929867321666140912155738]

75 **Nakajou K**, Watanabe H, Kragh-Hansen U, Maruyama T, Otagiri M. The effect of glycation on the structure, function and biological fate of human serum albumin as revealed by recombinant mutants. *Biochim Biophys Acta* 2003; **1623**: 88-97 [PMID: 14572906 DOI: 10.1016/j.bbagen.2003.08.001]

76 **Joseph KS**, Hage DS. The effects of glycation on the binding of human serum albumin to warfarin and L-tryptophan. *J Pharm Biomed Anal* 2010; **53**: 811-818 [PMID: 20537832 DOI: 10.1016/j.jpba.2010.04.035]

77 **Martinez Fernandez A**, Regazzoni L, Brioschi M, Gianazza E, Agostoni P, Aldini G, Banfi C. Pro-oxidant and pro-inflammatory effects of glycated albumin on cardiomyocytes. *Free Radic Biol Med* 2019; **144**: 245-255 [PMID: 31260731 DOI: 10.1016/j.freeradbiomed.2019.06.023]

78 **Szkudlarek A**, Sułkowska A, Maciążek-Jurczyk M, Chudzik M, Równicka-Zubik J. Effects of non-enzymatic glycation in human serum albumin. Spectroscopic analysis. *Spectrochim Acta A Mol Biomol Spectrosc* 2016; **152**: 645-653 [PMID: 25735846 DOI: 10.1016/j.saa.2015.01.120]

79 **Dockal M**, Carter DC, Rüker F. The three recombinant domains of human serum albumin. Structural characterization and ligand binding properties. *J Biol Chem* 1999; **274**: 29303-29310 [PMID: 10506189 DOI: 10.1074/jbc.274.41.29303]

80 **Krenzel ES**, Chen Z, Hamilton JA. Correspondence of fatty acid and drug binding sites on human serum albumin: a two-dimensional nuclear magnetic resonance study. *Biochemistry* 2013; **52**: 1559-1567 [PMID: 23360066 DOI: 10.1021/bi301458b]

81 **Guerin-Dubourg A**, Catan A, Bourdon E, Rondeau P. Structural modifications of human albumin in diabetes. *Diabetes Metab* 2012; **38**: 171-178 [PMID: 22349032 DOI: 10.1016/j.diabet.2011.11.002]

82 **Koizumi K**, Ikeda C, Ito M, Suzuki J, Kinoshita T, Yasukawa K, Hanai T. Influence of glycosylation on the drug binding of human serum albumin. *Biomed Chromatogr* 1998; **12**: 203-210 [PMID: 9667024 DOI: 10.1002/(SICI)1099-0801(199807/08)12:4<203::AID-BMC736>3.0.CO;2-L]

83 **Okabe N**, Hashizume N. Drug binding properties of glycosylated human serum albumin as measured by fluorescence and circular dichroism. *Biol Pharm Bull* 1994; **17**: 16-21 [PMID: 8148809 DOI: 10.1248/bpb.17.16]

84 **Yamazaki E**, Inagaki M, Kurita O, Inoue T. Kinetics of fatty acid binding ability of glycated human serum albumin. *J Biosci* 2005; **30**: 475-481 [PMID: 16184009 DOI: 10.1007/BF02703721]

85 **Karp WB**, Kinsley M, Subramanyam SB, Robertson AF. Binding properties of glycosylated albumin and acetaldehyde albumin. *Alcohol Clin Exp Res* 1985; **9**: 429-432 [PMID: 3904507 DOI: 10.1111/j.1530-0277.1985.tb05577.x]

86 **Brownlee M**. Glycosylation products as toxic mediators of diabetic complications. *Annu Rev Med* 1991; **42**: 159-166 [PMID: 2035962 DOI: 10.1146/annurev.me.42.020191.001111]

87 **Tanaka S**, Avigad G, Brodsky B, Eikenberry EF. Glycation induces expansion of the molecular packing of collagen. *J Mol Biol* 1988; **203**: 495-505 [PMID: 3143838 DOI: 10.1016/0022-2836(88)90015-0]

88 **Esposito C**, Gerlach H, Brett J, Stern D, Vlassara H. Endothelial receptor-mediated binding of glucose-modified albumin is associated with increased monolayer permeability and modulation of cell surface coagulant properties. *J Exp Med* 1989; **170**: 1387-1407 [PMID: 2551990 DOI: 10.1084/jem.170.4.1387]

89 **Vlassara H**, Brownlee M, Manogue KR, Dinarello CA, Pasagian A. Cachectin/TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodeling. *Science* 1988; **240**: 1546-1548 [PMID: 3259727 DOI: 10.1126/science.3259727]

90 **Baker L**, Dahlem S, Goldfarb S, Kern EF, Stanley CA, Egler J, Olshan JS, Heyman S. Hyperfiltration and renal disease in glycogen storage disease, type I. *Kidney Int* 1989; **35**: 1345-1350 [PMID: 2671467 DOI: 10.1038/ki.1989.133]

91 **Lapolla A**, Piarulli F, Sartore G, Ceriello A, Ragazzi E, Reitano R, Baccarin L, Laverda B, Fedele D. Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease. *Diabetes Care* 2007; **30**: 670-676 [PMID: 17327339 DOI: 10.2337/dc06-1508]

92 **Ramasamy R**, Yan SF, Schmidt AM. Arguing for the motion: yes, RAGE is a receptor for advanced glycation endproducts. *Mol Nutr Food Res* 2007; **51**: 1111-1115 [PMID: 17854009 DOI: 10.1002/mnfr.200700008]

93 **Roohk HV**, Zaidi AR. A review of glycated albumin as an intermediate glycation index for controlling diabetes. *J Diabetes Sci Technol* 2008; **2**: 1114-1121 [PMID: 19885300 DOI: 10.1177/193229680800200620]

94 **Koga M**, Kasayama S. Clinical impact of glycated albumin as another glycemic control marker. *Endocr J* 2010; **57**: 751-762 [PMID: 20724796 DOI: 10.1507/endocrj.k10e-138]

95 **Gillery P**. A history of HbA1c through Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med* 2013; **51**: 65-74 [PMID: 22992284 DOI: 10.1515/cclm-2012-0548]

96 **Bry L**, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on

assays for glycohemoglobin. *Clin Chem* 2001; **47**: 153-163 [PMID: 11159762]
97 **Jeffcoate SL**. Diabetes control and complications: the role of glycated haemoglobin, 25 years on. *Diabet Med* 2004; **21**: 657-665 [PMID: 15209755 DOI: 10.1046/j.1464-5491.2003.01065.x]
98 **Behan KJ**, Merschen J. HbA1c does not always estimate average glucose. *Clin Lab Sci* 2011; **24**: 71-77 [PMID: 21657138 DOI: 10.29074/ascls.24.2.71]
99 **Panzer S**, Kronik G, Lechner K, Bettelheim P, Neumann E, Dudczak R. Glycosylated hemoglobins (GHb): an index of red cell survival. *Blood* 1982; **59**: 1348-1350 [PMID: 7082831]
100 **Brooks AP**, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. *Lancet* 1980; **2**: 141 [PMID: 6105305 DOI: 10.1016/s0140-6736(80)90019-7]
101 **Kim PS**, Woods C, Georgoff P, Crum D, Rosenberg A, Smith M, Hadigan C. A1C underestimates glycemia in HIV infection. *Diabetes Care* 2009; **32**: 1591-1593 [PMID: 19502538 DOI: 10.2337/dc09-0177]
102 **Phelps RL**, Honig GR, Green D, Metzger BE, Frederiksen MC, Freinkel N. Biphasic changes in hemoglobin A1c concentrations during normal human pregnancy. *Am J Obstet Gynecol* 1983; **147**: 651-653 [PMID: 6605685 DOI: 10.1016/0002-9378(83)90443-x]
103 **Jiao Y**, Okumiya T, Saibara T, Park K, Sasaki M. Abnormally decreased HbA1c can be assessed with erythrocyte creatine in patients with a shortened erythrocyte age. *Diabetes Care* 1998; **21**: 1732-1735 [PMID: 9773739 DOI: 10.2337/diacare.21.10.1732]
104 **Coban E**, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. *Acta Haematol* 2004; **112**: 126-128 [PMID: 15345893 DOI: 10.1159/000079722]
105 **Lo C**, Lui M, Ranasinha S, Teede HJ, Kerr PG, Polkinghorne KR, Nathan DM, Zheng H, Zoungas S. Defining the relationship between average glucose and HbA1c in patients with type 2 diabetes and chronic kidney disease. *Diabetes Res Clin Pract* 2014; **104**: 84-91 [PMID: 24573088 DOI: 10.1016/j.diabres.2014.01.020]
106 **Seidel UK**, Gronewold J, Volsek M, Todica O, Kribben A, Bruck H, Hermann DM. The prevalence, severity, and association with HbA1c and fibrinogen of cognitive impairment in chronic kidney disease. *Kidney Int* 2014; **85**: 693-702 [PMID: 24088956 DOI: 10.1038/ki.2013.366]
107 **Vetter SW**. Glycated Serum Albumin and AGE Receptors. *Adv Clin Chem* 2015; **72**: 205-275 [PMID: 26471084 DOI: 10.1016/bs.acc.2015.07.005]
108 **Austin GE**, Mullins RH, Morin LG. Non-enzymic glycation of individual plasma proteins in normoglycemic and hyperglycemic patients. *Clin Chem* 1987; **33**: 2220-2224 [PMID: 3690840]
109 RJ, Beck RW, Scioscia MF, Umpierrez GE, Tuttle KR. Glycemic Monitoring and management in advanced chronic kidney disease. *Endocr Rev* 2020; **41**: 756-774 [PMID: 32455432 DOI: 10.1210/endrev/bnaa017]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1070-1080

DOI: 10.4239/wjd.v12.i7.1070

ISSN 1948-9358 (online)

MINIREVIEWS

Multi-omics: Opportunities for research on mechanism of type 2 diabetes mellitus

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Author contributions: Wang S was responsible for drafting the article; Yong H and He XD made contributions to data acquisition; He XD contributed to conception and provided final approval of the version of the article to be published.

Supported by Grant from International Joint Usage/Research Center, the Institute of Medical Science, the University of Tokyo, No. New-2020-K2012; and Open Project of Shandong Provincial Key Laboratory of Infection and Immunity, No. 2.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

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Abstract

Type 2 diabetes mellitus (T2DM) is a burdensome global disease. In-depth understanding of its mechanism will help to optimize diagnosis and treatment, which reduces the burden. Multi-omics research has unparalleled advantages in contributing to the overall understanding of the mechanism of this chronic metabolic disease. In the past two decades, the study of multi-omics on T2DM-related intestinal flora perturbation and plasma dyslipidemia has shown tremendous potential and is expected to achieve major breakthroughs. The regulation of intestinal flora in diabetic patients has been confirmed by multiple studies. The use of metagenomics, 16S RNA sequencing, and metabolomics has comprehensively identified the overall changes in the intestinal flora and the metabolic disturbances that could directly or indirectly participate in the intestinal flora-host interactions. Lipidomics combined with other "omics" has characterized lipid metabolism disorders in T2DM. The combined application and cross-validation of multi-omics can screen for dysregulation in T2DM, which will provide immense opportunities to understand the mechanisms behind T2DM.

Key Words: Type 2 diabetes mellitus; Gastrointestinal microbiome; Intestinal flora; Lipid metabolism disorders; Dyslipidemias; Metabolomics

Core Tip: The prospects of multi-omics in the study of the mechanisms of type 2 diabetes mellitus (T2DM)-related intestinal flora perturbation and plasma dyslipidemia are tremendous. The use of multi-omics has identified variations in T2DM intestinal

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 24, 2021
Peer-review started: January 24, 2021
First decision: March 16, 2021
Revised: March 22, 2021
Accepted: May 22, 2021
Article in press: May 22, 2021
Published online: July 15, 2021

P-Reviewer: Mazlan M
S-Editor: Gao CC
L-Editor: Wang TQ
P-Editor: Ma YJ

flora composition and human-microbiota interactions. However, further sequencing is required, and the clinical application needs to be clarified and simplified. Multi-omics is also identifying T2DM lipid profiles, which will provide immense opportunities to understand the mechanisms of T2DM-related dyslipidemia.

Citation: Wang S, Yong H, He XD. Multi-omics: Opportunities for research on mechanism of type 2 diabetes mellitus. *World J Diabetes* 2021; 12(7): 1070-1080
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1070.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1070

INTRODUCTION

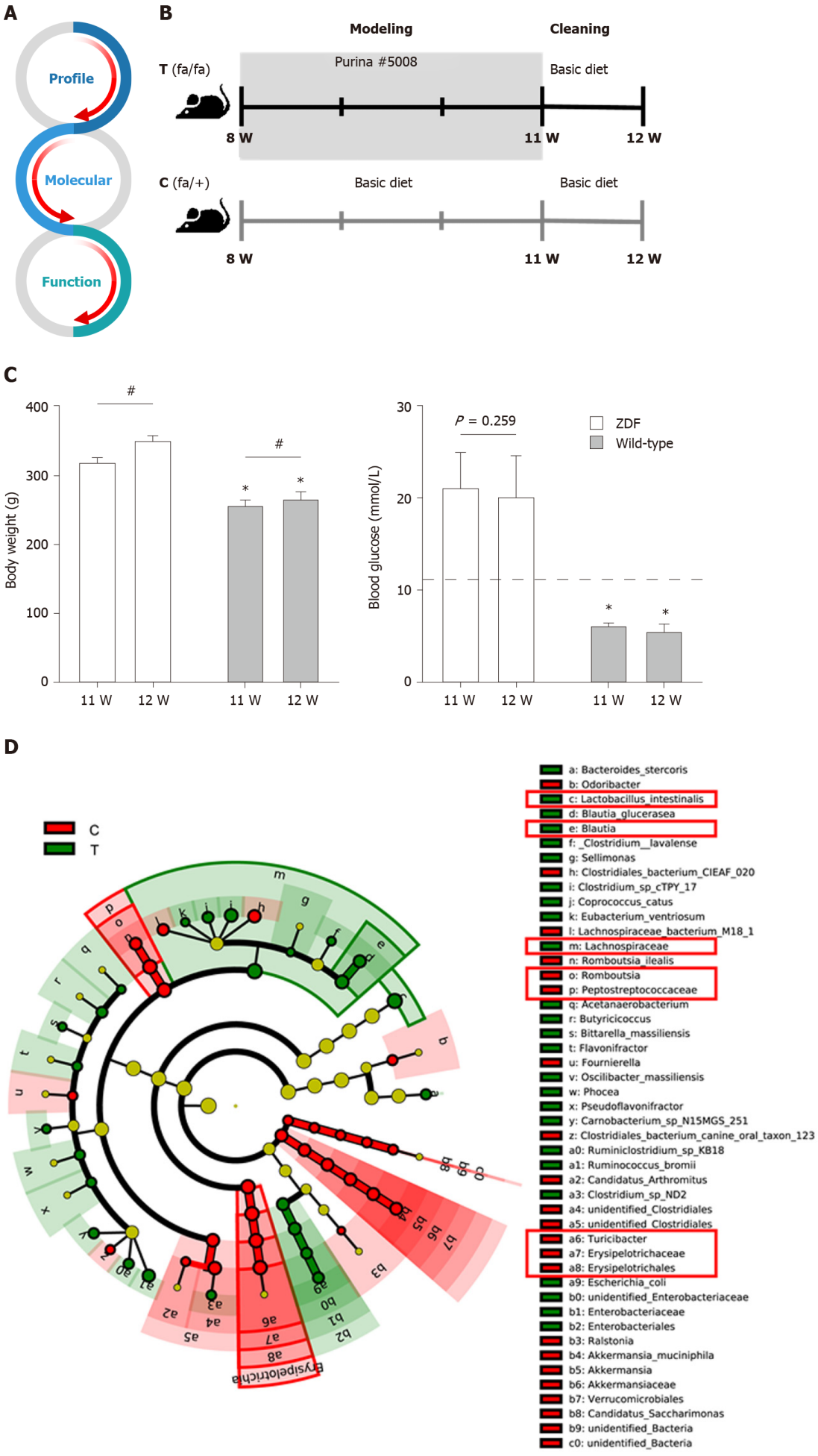
According to the World Health Organization, about 422 million people worldwide have diabetes and 1.6 million deaths are directly attributed to diabetes each year. Diabetes is a chronic, metabolic disease characterized by elevated blood glucose (or blood sugar) levels, which increases morbidity and mortality. When the body does not produce enough insulin or does not use it efficiently, diabetes manifests. The number of patients with diabetes is increasing, which expands the magnitude of the disease burden[1]. The most common type of diabetes is type 2 diabetes mellitus (T2DM)[2]. When genetics, age, and family history are fixed, reducing exposure to other known risk factors for T2DM using a variety of interventions and improving access to and quality of care decrease the incidence of T2DM and benefit patients with T2DM[3]. The effectiveness of these interventions depends largely on the understanding of the mechanism of T2DM. Due to the complexity of the mechanisms and causes of T2DM, traditional bench science has limitations. Multi-omics, including genomics, transcriptomics, proteomics, glycomics, metabolomics, epigenomics, ncRNomics, lipidomics, and interactomics, offers a fresh and exciting conceptual lens that will aid scientists to comprehensively and systematically understand the physiological processes and regulatory mechanisms of T2DM[4].

ADVANTAGES OF MULTI-OMICS ON STUDY OF MECHANISM OF T2DM

Since the concept of genomes and genomics was introduced, "omics" research has profoundly affected systems biology discoveries. Multi-omics research enables researchers to identify the differences in genes, proteins, and metabolites that lead to understanding overall functional disturbances in diseases, including T2DM.

Using multi-omics, our previous research[5,6] described the intestinal flora alterations and the plasma protein metabolic profile perturbations in the Zucker diabetic fatty rat, which is a spontaneous T2DM animal model commonly used to develop drugs for treating diabetes[7-10]. The altered intestinal microbiota and differentially expressed proteins and metabolites clearly distinguished the treatment group [T: Zucker leptin receptor gene-deficient rats (fa/fa) treated by Purina #5008] from the control group [C: basic diet-fed litter mate wild-type controls (fa/+)]. This provided an important reference for screening and verifying T2DM by utilizing intestinal flora and plasma biomarkers. Using "omics" techniques, the increased levels of glycated hemoglobin, ceruloplasmin, triacylglycerols, diacylglycerols, phosphatidylethanolamines, *etc.* in plasma/urine have been verified in the human population. They are gradually being introduced in the early diagnosis of diabetes and the prediction of serious adverse complications[11-13].

Further functional analysis of the differential molecules using multi-omics revealed the pathophysiological mechanism of T2DM (Figure 1). The intestinal flora and the host exhibit similar features that focus on oxidative stress, insulin resistance, and metabolic disorders. This data confirmed previous T2DM mechanism research[14-16] and provided insight into the overall levels of molecules.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a: Bacteroides_stercoris
b: Odoribacter
c: Lactobacillus_intestinalis
d: Blautia_glucerasea
e: Blautia
f: _Clostridium__lavalense
g: Sellimonas
h: Clostridiales_bacterium_CIEAF_020
i: Clostridium_sp_cTPY_17
j: Coprococcus_catus
k: Eubacterium_ventriosum
l: Lachnospiraceae_bacterium_M18_1
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n: Romboutsia_ilealis
o: Romboutsia
p: Peptostreptococcaceae
q: Acetanaerobacterium
r: Butyricicoccus
s: Bittarella_massiliensis
t: Flavonifractor
u: Fournierella
v: Oscilibacter_massiliensis
w: Phocea
x: Pseudoflavonifractor
y: Carnobacterium_sp_N15MGS_251
z: Clostridiales_bacterium_canine_oral_taxon_123
a0: Ruminiclostridium_sp_KB18
a1: Ruminococcus_bromii
a2: Candidatus_Arthromitus
a3: Clostridium_sp_ND2
a4: unidentified_Clostridiales
a5: unidentified_Clostridiales
a6: Turicibacter
a7: Erysipelotrichaceae
a8: Erysipelotrichales
a9: Escherichia_coli
b0: unidentified_Enterobacteriaceae
b1: Enterobacteriaceae
b2: Enterobacteriales
b3: Ralstonia
b4: Akkermansia_muciniphila
b5: Akkermansia
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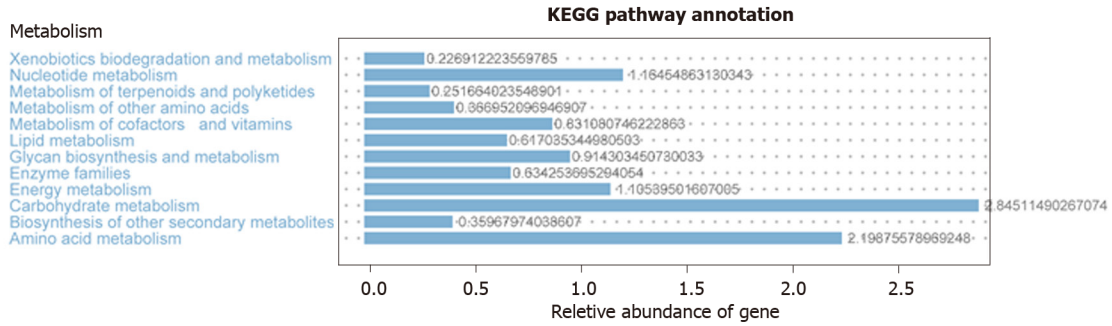
KEGG pathway annotation
Metabolism
Xenobiotics biodegradation and metabolism
Nucleotide metabolism
Metabolism of terpenoids and polyketides
Metabolism of other amino acids
Metabolism of cofactors and vitamins
Lipid metabolism
Glycan biosynthesis and metabolism
Enzyme families
Energy metabolism
Carbohydrate metabolism
Biosynthesis of other secondary metabolites
Amino acid metabolism
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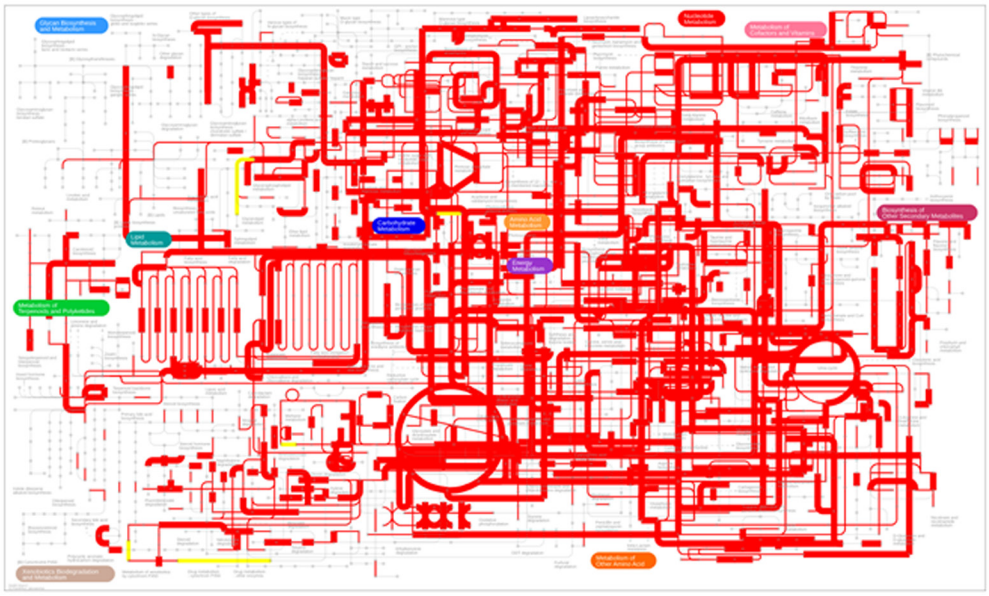

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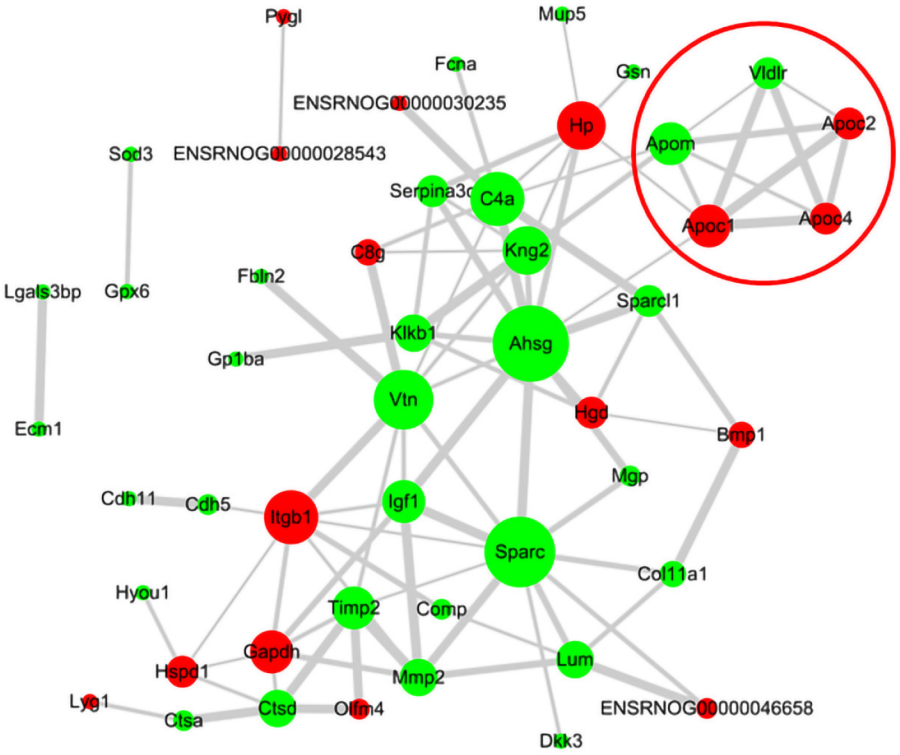
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C4a
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C8g
Kng2
Fbln2
Lgals3bp
Gpx6
Sparcl1
Klkb1
Ahsg
Gp1ba
Vtn
Hgd
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Hyou1
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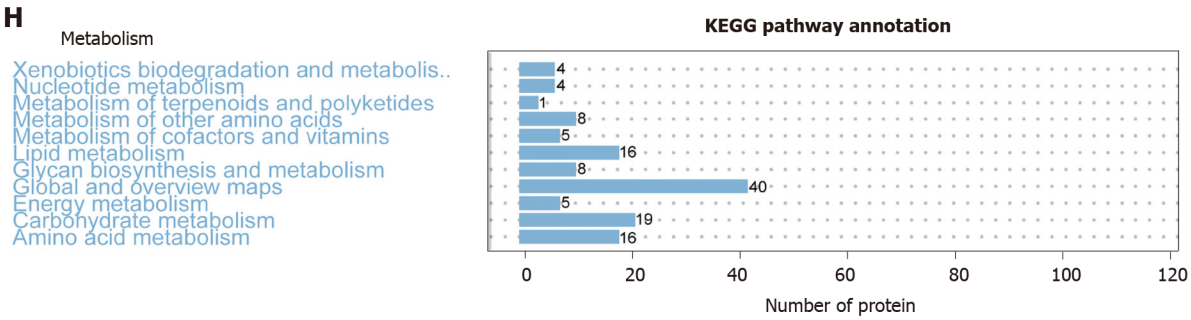

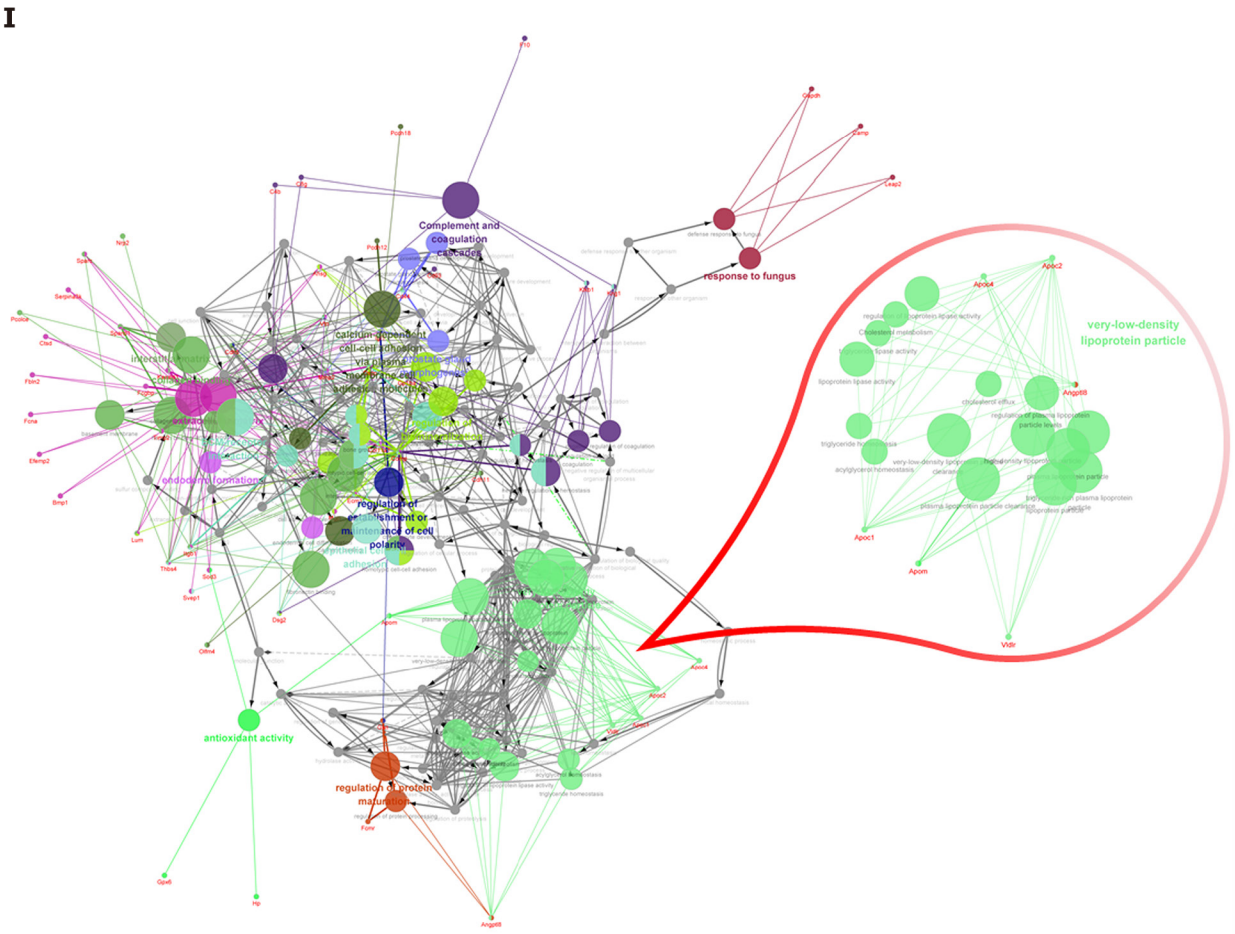

Figure 1 Multi-omics verification of the mechanism of type 2 diabetes mellitus. A: Schematic diagram of multi-omics verification; B: Conceptual diagram of the Zucker diabetic fatty rat modeling process; C: Body weight (left) and blood sugar (right) before and after basic diet feeding in B; D: Fecal 16S rRNA sequencing biomarkers; E: Genomic functions of the disturbed intestinal flora; F: Overview of the complete metabolism in the two biological system; G: Protein-protein interaction network of plasma differentially expressed proteins (DEPs); H: Functional enrichment of DEPs; I: Visualization of G and H. The cross-validation of intestinal flora and the plasma proteome further emphasizes that oxidative stress, insulin resistance, energy intake and consumption imbalances, and lipid metabolism disorders play an important part in the occurrence of type 2 diabetes mellitus (T2DM). Dyslipidemia may be a major hub for the *in vivo* and *in vitro* changes in T2DM (unpublished data). The data used in the figure are our published and public data in open access journals, which are displayed after further analysis. C and G: Citation: Wang S, Lu Z, Wang Y, Zhang T, He X. Metalloproteins and apolipoprotein C: candidate plasma biomarkers of T2DM screened by comparative proteomics and lipidomics in ZDF rats. Nutr Metab (Lond) 2020; 17: 66. Copyright ©The Author(s) 2020. Published by Springer Nature[6]. T: Zucker leptin receptor gene-deficient rats (fa/fa) treated by Purina #5008 for 3 wk; C: Basic diet-fed litter mate wild-type controls (fa/+); DEPs: Differentially expressed proteins; T2DM: Type 2 diabetes mellitus.

MULTI-OMICS AND T2DM-RELATED INTESTINAL FLORA DISTURBANCE

Multi-omics aided the dramatic discovery that diabetes was associated with the intestinal flora. The altered microbiota observed in genetically obese mice[17] or people[18-20] is sufficient to promote increased adiposity in lean mice that receive a microbiota transplant. Germ-free mice, which lack a microbiota, have reduced adiposity and improved tolerance to glucose and insulin when compared to their counterparts[21]. They are also free from diet-induced obesity when fed a Western-

style diet[22-24]. Taken together, the correlation between intestinal flora, obesity, and diabetes demonstrates that the microbiota contributes to the regulation of adiposity and T2DM[25]. However, the precise mechanism is still not clear.

Increased metagenomics data (usually 16S RNA sequencing) suggest that the extent of biodiversity within an ecosystem can be an important mechanism and serve as a measure of stability and robustness. In other words, a reduction in gut microbiome diversity and richness is linked to susceptibility to T2DM[26,27]. The complex composition of the intestinal flora can quickly change in response to a diverse diet, while simpler flora composition can only interact with specific diets and increase the vulnerability of the intestinal tract[28]. In T2DM rats and patients, the proportion of *Firmicutes* decreased and that of *Bacteroidetes* increased[29]. This ratio can be used as a simple indicator of intestinal flora diversity in T2DM. Although metagenomics has an irreplaceable advantage in T2DM-related intestinal flora studies, sequencing depth and defects in methods still need to be paid more attention. 16S RNA sequencing enables taxonomic identification to at least the family level but is rarely able to make a distinction between different strains of the same species or related species[30]. *Salmonella*, *Escherichia coli*, and *Shigella* would be identified by the same sequence, yet their significance in T2DM may vary.

Although the intestinal flora ratios may be important, the metabolic function of the intestinal flora on nutrients and food likely plays a more significant role[31,32]. Metabolites of the intestinal flora are involved in numerous functions. They can affect the absorption of nutrients (bile acid metabolism is closely related to lipid absorption [33,34]), and they can be absorbed to provide nutrients for the host (carbohydrates are fermented to form short-chain fatty acids to provide energy for the host[35-37]). Metabolites can be signaling molecules to regulate inflammation and immunity inter- or intra-intestinally. Bacterial L-tryptophan metabolites enhance the secretion of glucagon-like peptide-1[38-40]. Endotoxins (lipopolysaccharides) induce inflammation [41] and limit the autoimmune response[42,43]. Many metabolites produced due to the interaction of numerous species, the allocation of resources, and the dynamic response to perturbation within the gut may serve as potential T2DM biomarkers[44]. Table 1 briefly summarizes related gut microbiota metabolites and their interactions with the host in diabetes. The complementation of intestinal metabolomics and metagenomics will enhance the research on T2DM-related intestinal flora disturbance[45].

Discovering the interactions of the intestinal microbiota under complex conditions, such as diet[46], drugs[47], genetic background of the host[48] and colonizing flora [49], modification of the intestinal flora through antibiotics[50-54], probiotics, prebiotics[55-57], and fecal microbiota transplantation, and causal relationships in people and precision treatment[58] will become more accessible using multi-omics. The potential of understanding the mechanisms of diabetes using multi-omics is tremendous. However, the systematic examination of T2DM-related colonic flora studies in therapeutic and clinical application must still be completed.

An interesting example of fully understanding the mechanism of intestinal bacteria is *Akkermansia muciniphila* (*A. muciniphila*). *A. muciniphila* is a mucosal-dwelling anaerobe and the only known member of its genus. Animal and human data have indicated an inverse correlation between the intestinal abundance of *A. muciniphila* and obesity, dyslipidemia, and T2DM[59-61]. *A. muciniphila* supplementation restores epithelial mucosal integrity, reduces weight gain and fat accumulation, improves glucose tolerance, and reduces inflammation and metabolic endotoxemia in animal models. It may be a potential treatment for T2DM. However, *A. muciniphila* levels are increased in patients with Parkinson's disease, multiple sclerosis, or Alzheimer's disease[62-64], suggesting that this bacterium may have unforeseen harmful effects on the nervous system. Pure culture, sterilization, and component extraction can reduce this risk[65], which is consistent with the recently proposed concept of culturomics [66]. In short, through multi-omics it is likely that major breakthroughs in the study of the mechanism of T2DM-related intestinal flora perturbation will be made and that these breakthroughs will be gradually applied in the clinic.

MULTI-OMICS AND T2DM-RELATED DYSLIPIDEMIA

While intestinal flora disorder is a diet-related *in vitro* regulatory mechanism of T2DM, glucose and lipid metabolism disorders and insulin resistance are *in vivo* mechanisms [67]. In addition to glycomics[68,69], we want to emphasize the role of lipidomics in T2DM-related dyslipidemia. Dyslipidemia in T2DM, characterized by a high concentration of triglycerides, low concentration of high-density lipoprotein cholesterol, and

Table 1 Intestinal flora-related metabolites and their host interaction mechanism

Metabolites	Potential interaction mechanism between intestinal flora-related metabolites and the host
Bile acids	Promotes fat absorption; serves as signaling molecule [acts with G-protein-coupled bile acid receptor 1 (Gpbar1, TGR5) and the bile acid receptor FXR]; limits the autoimmune response
SCFA	
Acetate and butyrate	Acts as histone deacetylase inhibitors; ameliorates inflammation
Propionate	Participates in carbohydrate esterification
Valeric	Provides calories; affects inflammation; enteroendocrine regulation through G-protein-coupled receptors (*e.g.*, GPR41, GPR43)
Indole	Promotes the function of the intestinal cell epithelial barrier; enhances the secretion of glucagon-like peptide-1 (GLP-1)
Endotoxins (LPS)	Induces inflammation; limits the autoimmune response
H_2S	Destroys the intestinal barrier function
TMA	Interferes with metabolism

Gpbar1 (TGR5): G-protein-coupled bile acid receptor 1; FXR: Farnesoid X receptor; SCFA: Short-chain fatty acid; GPR: G-protein-coupled receptors; GLP-1: Glucagon-like peptide-1; LPS: Lipopolysaccharide; H_2S: Hydrogen sulfide; TMA: Trimethylamine.

increased concentration of small, dense low-density lipoprotein cholesterol particles, is associated with insulin resistance. It is also one of the major risk factors for cardiovascular disease in patients with diabetes[70]. Although active control of triglycerides and low-density lipoprotein cholesterol can delay the progression of T2DM and reduce the risk of adverse cardiovascular outcomes in patients, interventions to increase high-density lipoprotein cholesterol have had little success. Niacin was thought to raise high-density lipoprotein cholesterol and was used to control diabetic dyslipidemia previously. However, it was proven to be ineffective and removed from the treatment guidelines[71-75]. Lipidomics, the systematic analysis of lipid composition and expression changes, can intensify the understanding of lipid metabolism alterations in T2DM. Recent population lipidomics data revealed that the T2DM-related lipid profile included decreased lysophospholipids, phosphatidylcholines, sphingomyelins, and cholesterol esters and increased triacylglycerols, diacylglycerols, and phosphatidylethanolamines[76-78]. Although these indicators can be used as potential biomarkers to predict the risk of T2DM, its prediction for a particular disease has not yet been verified. Balgoma *et al*[79] compared the lipid profiles from patients with non-alcoholic fatty liver disease, cardiovascular incidents, hepatocellular carcinoma, and T2DM. They noted that the upregulation of triacylglycerols, palmitic acid, palmitoleic acid, stearic acid and oleic acid is a fingerprint of liver X receptor-mediated lipogenesis in the liver. To thoroughly understand the mechanisms of T2DM-related lipid metabolism disorders, the utilization of lipidomics, and the integration of databases will undoubtedly achieve this goal.

CONCLUSION

Multi-omic studies have provided new breakthroughs and directions to guide traditional molecular biology research. The expansion of "omics" data and the continuous advancement of bioinformatics analysis technology will surely continue the advancement of our knowledge on the mechanisms of T2DM, especially on intestinal flora perturbation and dyslipidemia.

ACKNOWLEDGEMENTS

The authors want to thank Ms. Wang SE and Ms. Liu S for their suggestions and discussions during the writing process.

REFERENCES

1 **Saeedi P**, Salpea P, Karuranga S, Petersohn I, Malanda B, Gregg EW, Unwin N, Wild SH, Williams R. Mortality attributable to diabetes in 20-79 years old adults, 2019 estimates: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2020; **162**: 108086 [PMID: 32068099 DOI: 10.1016/j.diabres.2020.108086]

2 **Cole AR**, Astell A, Green C, Sutherland C. Molecular connexions between dementia and diabetes. *Neurosci Biobehav Rev* 2007; **31**: 1046-1063 [PMID: 17544131 DOI: 10.1016/j.neubiorev.2007.04.004]

3 **Chatterjee S**, Khunti K, Davies MJ. Type 2 diabetes. *Lancet* 2017; **389**: 2239-2251 [PMID: 28190580 DOI: 10.1016/S0140-6736(17)30058-2]

4 **Wang N**, Zhu F, Chen L, Chen K. Proteomics, metabolomics and metagenomics for type 2 diabetes and its complications. *Life Sci* 2018; **212**: 194-202 [PMID: 30243649 DOI: 10.1016/j.lfs.2018.09.035]

5 **Wang Y**, Ouyang M, Gao X, Wang S, Fu C, Zeng J, He X. *Phocea, Pseudoflavonifractor* and *Lactobacillus intestinalis*: Three Potential Biomarkers of Gut Microbiota That Affect Progression and Complications of Obesity-Induced Type 2 Diabetes Mellitus. *Diabetes Metab Syndr Obes* 2020; **13**: 835-850 [PMID: 32256098 DOI: 10.2147/DMSO.S240728]

6 **Wang S**, Lu Z, Wang Y, Zhang T, He X. Metalloproteins and apolipoprotein C: candidate plasma biomarkers of T2DM screened by comparative proteomics and lipidomics in ZDF rats. *Nutr Metab (Lond)* 2020; **17**: 66 [PMID: 32817751 DOI: 10.1186/s12986-020-00488-2]

7 **Ohneda M**, Inman LR, Unger RH. Caloric restriction in obese pre-diabetic rats prevents beta-cell depletion, loss of beta-cell GLUT 2 and glucose incompetence. *Diabetologia* 1995; **38**: 173-179 [PMID: 7713311 DOI: 10.1007/BF00400091]

8 **Cefalu WT**. Animal models of type 2 diabetes: clinical presentation and pathophysiological relevance to the human condition. *ILAR J* 2006; **47**: 186-198 [PMID: 16804194 DOI: 10.1093/ilar.47.3.186]

9 **Nugent DA**, Smith DM, Jones HB. A review of islet of Langerhans degeneration in rodent models of type 2 diabetes. *Toxicol Pathol* 2008; **36**: 529-551 [PMID: 18467681 DOI: 10.1177/0192623308318209]

10 **Al-Awar A**, Kupai K, Veszelka M, Szűcs G, Attieh Z, Murlasits Z, Török S, Pósa A, Varga C. Experimental Diabetes Mellitus in Different Animal Models. *J Diabetes Res* 2016; **2016**: 9051426 [PMID: 27595114 DOI: 10.1155/2016/9051426]

11 **Hameed A**, Mojsak P, Buczynska A, Suleria HAR, Kretowski A, Ciborowski M. Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM. *J Clin Med* 2020; **9** [PMID: 32708684 DOI: 10.3390/jcm9072257]

12 **Ge S**, Wang Y, Song M, Li X, Yu X, Wang H, Wang J, Zeng Q, Wang W. Type 2 Diabetes Mellitus: Integrative Analysis of Multiomics Data for Biomarker Discovery. *OMICS* 2018; **22**: 514-523 [PMID: 30004843 DOI: 10.1089/omi.2018.0053]

13 **Kim SW**, Choi JW, Yun JW, Chung IS, Cho HC, Song SE, Im SS, Song DK. Proteomics approach to identify serum biomarkers associated with the progression of diabetes in Korean patients with abdominal obesity. *PLoS One* 2019; **14**: e0222032 [PMID: 31504048 DOI: 10.1371/journal.pone.0222032]

14 **Wada J**, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. *Clin Sci (Lond)* 2013; **124**: 139-152 [PMID: 23075333 DOI: 10.1042/CS20120198]

15 **Mohamed J**, Nazratun Nafizah AH, Zariyantey AH, Budin SB. Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation. *Sultan Qaboos Univ Med J* 2016; **16**: e132-e141 [PMID: 27226903 DOI: 10.18295/squmj.2016.16.02.002]

16 **Rehman K**, Akash MSH. Mechanism of Generation of Oxidative Stress and Pathophysiology of Type 2 Diabetes Mellitus: How Are They Interlinked? *J Cell Biochem* 2017; **118**: 3577-3585 [PMID: 28460155 DOI: 10.1002/jcb.26097]

17 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]

18 **Le Chatelier E**, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T; MetaHIT consortium, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**: 541-546 [PMID: 23985870 DOI: 10.1038/nature12506]

19 **Ridaura VK**, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013; **341**: 1241214 [PMID: 24009397 DOI: 10.1126/science.1241214]

20 **Goodrich JK**, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE. Human genetics shape the gut microbiome. *Cell* 2014; **159**: 789-799 [PMID: 25417156 DOI: 10.1016/j.cell.2014.09.053]

21 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**:

15718-15723 [PMID: 15505215 DOI: 10.1073/pnas.0407076101]
22 **Bäckhed F**, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 2007; **104**: 979-984 [PMID: 17210919 DOI: 10.1073/pnas.0605374104]
23 **Rabot S**, Membrez M, Bruneau A, Gérard P, Harach T, Moser M, Raymond F, Mansourian R, Chou CJ. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J* 2010; **24**: 4948-4959 [PMID: 20724524 DOI: 10.1096/fj.10-164921]
24 **Ding S**, Chi MM, Scull BP, Rigby R, Schwerbrock NM, Magness S, Jobin C, Lund PK. High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PLoS One* 2010; **5**: e12191 [PMID: 20808947 DOI: 10.1371/journal.pone.0012191]
25 **Navab-Moghadam F**, Sedighi M, Khamseh ME, Alaei-Shahmiri F, Talebi M, Razavi S, Amirmozafari N. The association of type II diabetes with gut microbiota composition. *Microb Pathog* 2017; **110**: 630-636 [PMID: 28739439 DOI: 10.1016/j.micpath.2017.07.034]
26 **Cardinale BJ**, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S. Biodiversity loss and its impact on humanity. *Nature* 2012; **486**: 59-67 [PMID: 22678280 DOI: 10.1038/nature11148]
27 **Vallianou N**, Stratigou T, Christodoulatos GS, Dalamaga M. Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. *Curr Obes Rep* 2019; **8**: 317-332 [PMID: 31175629 DOI: 10.1007/s13679-019-00352-2]
28 **Wu GD**, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**: 105-108 [PMID: 21885731 DOI: 10.1126/science.1208344]
29 **Larsen N**, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010; **5**: e9085 [PMID: 20140211 DOI: 10.1371/journal.pone.0009085]
30 **Cirstea M**, Radisavljevic N, Finlay BB. Good Bug, Bad Bug: Breaking through Microbial Stereotypes. *Cell Host Microbe* 2018; **23**: 10-13 [PMID: 29324224 DOI: 10.1016/j.chom.2017.12.008]
31 **Rowland I**, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* 2018; **57**: 1-24 [PMID: 28393285 DOI: 10.1007/s00394-017-1445-8]
32 **Pascale A**, Marchesi N, Marelli C, Coppola A, Luzi L, Govoni S, Giustina A, Gazzaruso C. Microbiota and metabolic diseases. *Endocrine* 2018; **61**: 357-371 [PMID: 29721802 DOI: 10.1007/s12020-018-1605-5]
33 **Kawamata Y**, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, Hinuma S, Fujisawa Y, Fujino M. A G protein-coupled receptor responsive to bile acids. *J Biol Chem* 2003; **278**: 9435-9440 [PMID: 12524422 DOI: 10.1074/jbc.M209706200]
34 **Thomas C**, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov* 2008; **7**: 678-693 [PMID: 18670431 DOI: 10.1038/nrd2619]
35 **McNeil NI**. The contribution of the large intestine to energy supplies in man. *Am J Clin Nutr* 1984; **39**: 338-342 [PMID: 6320630 DOI: 10.1093/ajcn/39.2.338]
36 **Bergman EN**. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 1990; **70**: 567-590 [PMID: 2181501 DOI: 10.1152/physrev.1990.70.2.567]
37 **Mandaliya DK**, Seshadri S. Short Chain Fatty Acids, pancreatic dysfunction and type 2 diabetes. *Pancreatology* 2019; **19**: 280-284 [PMID: 30713129 DOI: 10.1016/j.pan.2019.01.021]
38 **Miele L**, Giorgio V, Alberelli MA, De Candia E, Gasbarrini A, Grieco A. Impact of Gut Microbiota on Obesity, Diabetes, and Cardiovascular Disease Risk. *Curr Cardiol Rep* 2015; **17**: 120 [PMID: 26497040 DOI: 10.1007/s11886-015-0671-z]
39 **de Mello VD**, Paananen J, Lindström J, Lankinen MA, Shi L, Kuusisto J, Pihlajamäki J, Auriola S, Lehtonen M, Rolandsson O, Bergdahl IA, Nordin E, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Landberg R, Eriksson JG, Tuomilehto J, Hanhineva K, Uusitupa M. Indolepropionic acid and novel lipid metabolites are associated with a lower risk of type 2 diabetes in the Finnish Diabetes Prevention Study. *Sci Rep* 2017; **7**: 46337 [PMID: 28397877 DOI: 10.1038/srep46337]
40 **Gao J**, Xu K, Liu H, Liu G, Bai M, Peng C, Li T, Yin Y. Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. *Front Cell Infect Microbiol* 2018; **8**: 13 [PMID: 29468141 DOI: 10.3389/fcimb.2018.00013]
41 **Caesar R**, Reigstad CS, Bäckhed HK, Reinhardt C, Ketonen M, Lundén GÖ, Cani PD, Bäckhed F. Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. *Gut* 2012; **61**: 1701-1707 [PMID: 22535377 DOI: 10.1136/gutjnl-2011-301689]
42 **Saito T**, Hayashida H, Furugen R. Comment on: Cani *et al* (2007) Metabolic endotoxemia initiates obesity and insulin resistance: Diabetes 56:1761-1772. *Diabetes* 2007; **56**: e20; author reply e21

[PMID: 18042755 DOI: 10.2337/db07-1181]
43 **Erridge C**, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am J Clin Nutr* 2007; **86**: 1286-1292 [PMID: 17991637 DOI: 10.1093/ajcn/86.5.1286]
44 **Sonnenburg JL**, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature* 2016; **535**: 56-64 [PMID: 27383980 DOI: 10.1038/nature18846]
45 **Peng W**, Huang J, Yang J, Zhang Z, Yu R, Fayyaz S, Zhang S, Qin YH. Integrated 16S rRNA Sequencing, Metagenomics, and Metabolomics to Characterize Gut Microbial Composition, Function, and Fecal Metabolic Phenotype in Non-obese Type 2 Diabetic Goto-Kakizaki Rats. *Front Microbiol* 2019; **10**: 3141 [PMID: 32038574 DOI: 10.3389/fmicb.2019.03141]
46 **David LA**, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; **505**: 559-563 [PMID: 24336217 DOI: 10.1038/nature12820]
47 **Rodriguez J**, Hiel S, Delzenne NM. Metformin: old friend, new ways of action-implication of the gut microbiome? *Curr Opin Clin Nutr Metab Care* 2018; **21**: 294-301 [PMID: 29634493 DOI: 10.1097/MCO.0000000000000468]
48 **Pryor R**, Norvaisas P, Marinos G, Best L, Thingholm LB, Quintaneiro LM, De Haes W, Esser D, Waschina S, Lujan C, Smith RL, Scott TA, Martinez-Martinez D, Woodward O, Bryson K, Laudes M, Lieb W, Houtkooper RH, Franke A, Temmerman L, Bjedov I, Cochemé HM, Kaleta C, Cabreiro F. Host-Microbe-Drug-Nutrient Screen Identifies Bacterial Effectors of Metformin Therapy. *Cell* 2019; **178**: 1299-1312. e29 [PMID: 31474368 DOI: 10.1016/j.cell.2019.08.003]
49 **Lee SM**, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. *Nature* 2013; **501**: 426-429 [PMID: 23955152 DOI: 10.1038/nature12447]
50 **Membrez M**, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, Corthesy I, Macé K, Chou CJ. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J* 2008; **22**: 2416-2426 [PMID: 18326786 DOI: 10.1096/fj.07-102723]
51 **Chou CJ**, Membrez M, Blancher F. Gut decontamination with norfloxacin and ampicillin enhances insulin sensitivity in mice. *Nestle Nutr Workshop Ser Pediatr Program* 2008; **62**: 127-137; discussion 137 [PMID: 18626197 DOI: 10.1159/000146256]
52 **Han J**, Lin H, Huang W. Modulating gut microbiota as an anti-diabetic mechanism of berberine. *Med Sci Monit* 2011; **17**: RA164-RA167 [PMID: 21709646 DOI: 10.12659/msm.881842]
53 **Thuny F**, Richet H, Casalta JP, Angelakis E, Habib G, Raoult D. Vancomycin treatment of infective endocarditis is linked with recently acquired obesity. *PLoS One* 2010; **5**: e9074 [PMID: 20161775 DOI: 10.1371/journal.pone.0009074]
54 **Hernández E**, Bargiela R, Diez MS, Friedrichs A, Pérez-Cobas AE, Gosalbes MJ, Knecht H, Martínez-Martínez M, Seifert J, von Bergen M, Artacho A, Ruiz A, Campoy C, Latorre A, Ott SJ, Moya A, Suárez A, Martins dos Santos VA, Ferrer M. Functional consequences of microbial shifts in the human gastrointestinal tract linked to antibiotic treatment and obesity. *Gut Microbes* 2013; **4**: 306-315 [PMID: 23782552 DOI: 10.4161/gmic.25321]
55 **Yadav H**, Jain S, Sinha PR. Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. *Nutrition* 2007; **23**: 62-68 [PMID: 17084593 DOI: 10.1016/j.nut.2006.09.002]
56 **Moroti C**, Souza Magri LF, de Rezende Costa M, Cavallini DC, Sivieri K. Effect of the consumption of a new symbiotic shake on glycemia and cholesterol levels in elderly people with type 2 diabetes mellitus. *Lipids Health Dis* 2012; **11**: 29 [PMID: 22356933 DOI: 10.1186/1476-511X-11-29]
57 **Ejtahed HS**, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition* 2012; **28**: 539-543 [PMID: 22129852 DOI: 10.1016/j.nut.2011.08.013]
58 **Leustean AM**, Ciocoiu M, Sava A, Costea CF, Floria M, Tarniceriu CC, Tanase DM. Implications of the Intestinal Microbiota in Diagnosing the Progression of Diabetes and the Presence of Cardiovascular Complications. *J Diabetes Res* 2018; **2018**: 5205126 [PMID: 30539026 DOI: 10.1155/2018/5205126]
59 **Derrien M**, Belzer C, de Vos WM. Akkermansia muciniphila and its role in regulating host functions. *Microb Pathog* 2017; **106**: 171-181 [PMID: 26875998 DOI: 10.1016/j.micpath.2016.02.005]
60 **Zhai Q**, Feng S, Arjan N, Chen W. A next generation probiotic, *Akkermansia muciniphila*. *Crit Rev Food Sci Nutr* 2019; **59**: 3227-3236 [PMID: 30373382 DOI: 10.1080/10408398.2018.1517725]
61 **Xu Y**, Wang N, Tan HY, Li S, Zhang C, Feng Y. Function of *Akkermansia muciniphila* in Obesity: Interactions With Lipid Metabolism, Immune Response and Gut Systems. *Front Microbiol* 2020; **11**: 219 [PMID: 32153527 DOI: 10.3389/fmicb.2020.00219]
62 **Heintz-Buschart A**, Pandey U, Wicke T, Sixel-Döring F, Janzen A, Sittig-Wiegand E, Trenkwalder C, Oertel WH, Mollenhauer B, Wilmes P. The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. *Mov Disord* 2018; **33**: 88-98 [PMID: 28843021 DOI: 10.1002/mds.27105]
63 **Hill-Burns EM**, Debelius JW, Morton JT, Wissemann WT, Lewis MR, Wallen ZD, Peddada SD, Factor SA, Molho E, Zabetian CP, Knight R, Payami H. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Mov Disord* 2017; **32**: 739-749 [PMID: 28195358 DOI: 10.1002/mds.26942]

64 **Cekanaviciute E**, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL, Crabtree-Hartman E, Sand IK, Gacias M, Zhu Y, Casaccia P, Cree BAC, Knight R, Mazmanian SK, Baranzini SE. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci USA* 2017; **114**: 10713-10718 [PMID: 28893978 DOI: 10.1073/pnas.1711235114]

65 **Plovier H**, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chilloux J, Ottman N, Duparc T, Lichtenstein L, Myridakis A, Delzenne NM, Klievink J, Bhattacharjee A, van der Ark KC, Aalvink S, Martinez LO, Dumas ME, Maiter D, Loumaye A, Hermans MP, Thissen JP, Belzer C, de Vos WM, Cani PD. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017; **23**: 107-113 [PMID: 27892954 DOI: 10.1038/nm.4236]

66 **Kambouris ME**, Pavlidis C, Skoufas E, Arabatzis M, Kantzanou M, Velegraki A, Patrinos GP. Culturomics: A New Kid on the Block of OMICS to Enable Personalized Medicine. *OMICS* 2018; **22**: 108-118 [PMID: 28402209 DOI: 10.1089/omi.2017.0017]

67 **Tomkin GH**. Dyslipidaemia--hepatic and intestinal cross-talk. *Atheroscler Suppl* 2010; **11**: 5-9 [PMID: 20434963 DOI: 10.1016/j.atherosclerosissup.2010.03.005]

68 **Liu J**, Dolikun M, Štambuk J, Trbojević-Akmačić I, Zhang J, Wang H, Meng X, Razdorov G, Menon D, Zheng D, Wu L, Wang Y, Song M, Lauc G, Wang W. Glycomics for Type 2 Diabetes Biomarker Discovery: Promise of Immunoglobulin G Subclass-Specific Fragment Crystallizable N-glycosylation in the Uyghur Population. *OMICS* 2019; **23**: 640-648 [PMID: 31393219 DOI: 10.1089/omi.2019.0052]

69 **Kunej T**. Rise of Systems Glycobiology and Personalized Glycomedicine: Why and How to Integrate Glycomics with Multiomics Science? *OMICS* 2019; **23**: 615-622 [PMID: 31651212 DOI: 10.1089/omi.2019.0149]

70 **Mooradian AD**. Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract Endocrinol Metab* 2009; **5**: 150-159 [PMID: 19229235 DOI: 10.1038/ncpendmet1066]

71 **HPS2-THRIVE Collaborative Group**, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, Wallendszus K, Craig M, Jiang L, Collins R, Armitage J. Effects of extended-release niacin with laropiprant in high-risk patients. *N Engl J Med* 2014; **371**: 203-212 [PMID: 25014686 DOI: 10.1056/NEJMoa1300955]

72 **Tuteja S**, Rader DJ. Dyslipidaemia: cardiovascular prevention--end of the road for niacin? *Nat Rev Endocrinol* 2014; **10**: 646-647 [PMID: 25178730 DOI: 10.1038/nrendo.2014.159]

73 **Mani P**, Rohatgi A. Niacin Therapy, HDL Cholesterol, and Cardiovascular Disease: Is the HDL Hypothesis Defunct? *Curr Atheroscler Rep* 2015; **17**: 43 [PMID: 26048725 DOI: 10.1007/s11883-015-0521-x]

74 **Kent S**, Haynes R, Hopewell JC, Parish S, Gray A, Landray MJ, Collins R, Armitage J, Mihaylova B; HPS2-THRIVE Collaborative Group. Effects of Vascular and Nonvascular Adverse Events and of Extended-Release Niacin With Laropiprant on Health and Healthcare Costs. *Circ Cardiovasc Qual Outcomes* 2016; **9**: 348-354 [PMID: 27407053 DOI: 10.1161/CIRCOUTCOMES.115.002592]

75 **Haynes R**, Valdes-Marquez E, Hopewell JC, Chen F, Li J, Parish S, Landray MJ, Armitage J; HPS2-THRIVE Collaborative Group; HPS2-THRIVE Writing Committee members; HPS2-THRIVE Steering Committee members. Serious Adverse Effects of Extended-release Niacin/Laropiprant: Results From the Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) Trial. *Clin Ther* 2019; **41**: 1767-1777 [PMID: 31447131 DOI: 10.1016/j.clinthera.2019.06.012]

76 **Razquin C**, Toledo E, Clish CB, Ruiz-Canela M, Dennis C, Corella D, Papandreou C, Ros E, Estruch R, Guasch-Ferré M, Gómez-Gracia E, Fitó M, Yu E, Lapetra J, Wang D, Romaguera D, Liang L, Alonso-Gómez A, Deik A, Bullo M, Serra-Majem L, Salas-Salvadó J, Hu FB, Martínez-González MA. Plasma Lipidomic Profiling and Risk of Type 2 Diabetes in the PREDIMED Trial. *Diabetes Care* 2018; **41**: 2617-2624 [PMID: 30327364 DOI: 10.2337/dc18-0840]

77 **Suvitaival T**, Bondia-Pons I, Yetukuri L, Pöhö P, Nolan JJ, Hyötyläinen T, Kuusisto J, Orešič M. Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men. *Metabolism* 2018; **78**: 1-12 [PMID: 28941595 DOI: 10.1016/j.metabol.2017.08.014]

78 **Mousa A**, Naderpoor N, Mellett N, Wilson K, Plebanski M, Meikle PJ, de Courten B. Lipidomic profiling reveals early-stage metabolic dysfunction in overweight or obese humans. *Biochim Biophys Acta Mol Cell Biol Lipids* 2019; **1864**: 335-343 [PMID: 30586632 DOI: 10.1016/j.bbalip.2018.12.014]

79 **Balgoma D**, Pettersson C, Hedeland M. Common Fatty Markers in Diseases with Dysregulated Lipogenesis. *Trends Endocrinol Metab* 2019; **30**: 283-285 [PMID: 30926249 DOI: 10.1016/j.tem.2019.02.008]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1081-1092

DOI: 10.4239/wjd.v12.i7.1081

ISSN 1948-9358 (online)

MINIREVIEWS

Role and function of granin proteins in diabetes mellitus

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Author contributions: Herold Z and Doleschall M searched the literature and drafted initial manuscript; Somogyi A provided further editing and comments; all authors have read and agreed to the published version of the manuscript.

Supported by the UNKP-20-4-I New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

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Abstract

The granin glycoprotein family consists of nine acidic proteins; chromogranin A (CgA), chromogranin B (CgB), and secretogranin II–VIII. They are produced by a wide range of neuronal, neuroendocrine, and endocrine cells throughout the human body. Their major intracellular function is to sort peptides and proteins into secretory granules, but their cleavage products also take part in the extracellular regulation of diverse biological processes. The contribution of granins to carbohydrate metabolism and diabetes mellitus is a recent research area. CgA is associated with glucose homeostasis and the progression of type 1 diabetes. WE-14, CgA_{10-19}, and CgA_{43-52} are peptide derivates of CgA, and act as $CD4^+$ or $CD8^+$ autoantigens in type 1 diabetes, whereas pancreastatin (PST) and catestatin have regulatory effects in carbohydrate metabolism. Furthermore, PST is related to gestational and type 2 diabetes. CgB has a crucial role in physiological insulin secretion. Secretogranins II and III have angiogenic activity in diabetic retinopathy (DR), and are novel targets in recent DR studies. Ongoing studies are beginning to investigate the potential use of granin derivatives as drugs to treat diabetes based on the divergent relationships between granins and different types of diabetes.

Key Words: Granin; Chromogranin A; Chromogranin B; Diabetes Mellitus; Mice; Inbred nonobese diabetic; Secretogranin III

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Hungary

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 27, 2021
Peer-review started: January 27, 2021
First decision: February 25, 2021
Revised: March 5, 2021
Accepted: May 17, 2021
Article in press: May 17, 2021
Published online: July 15, 2021

P-Reviewer: Liu D
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Liu JH

Core Tip: Granin glycoproteins are secretory proteins that are widely produced by neuronal, neuroendocrine, and endocrine cells throughout the human body. Recent data have shown that the granin proteins chromogranin A and B, and secretogranin II and III play a role in carbohydrate metabolism and in the pathophysiology of diabetes mellitus. In this review, the current state of knowledge concerning the relationship between granin proteins, diabetes and glucose homeostasis is discussed in detail, including several ongoing studies investigating granin-based drug therapies of future promise in diabetes care.

Citation: Herold Z, Doleschall M, Somogyi A. Role and function of granin proteins in diabetes mellitus. *World J Diabetes* 2021; 12(7): 1081-1092
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1081.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1081

INTRODUCTION

Neuronal, neuroendocrine, and endocrine cells are involved in the production of many peptides and proteins with diverse functions. During the secretion of these biologically active molecules, secretory proteins play an important role in the protein sorting that takes place in the secretory vesicles of the Golgi apparatus. The members belonging to the granin glycoprotein family, chromogranin A (CgA), chromogranin B (CgB), and secretogranin (Sg) II–VIII (Table 1), participate in protein sorting[1]. Granin proteins all have an acidic pH, calcium-binding ability, and are produced throughout the body by several types of neuronal-, neuroendocrine-, and endocrine cells[1-4]. In addition to protein sorting, secondary functions that are related mainly to the cleavage products of the granin proteins (Table 2) have emerged during evolution. Some of these biologically active products have been described as participating in pathogen control, psychiatric disorders, and metabolic disorders such as diabetes mellitus[1,3,5-7]. The function of many other granin protein products is still unclear. The available literature on granin proteins and their cleavage products is discussed in this review, focusing on their relationships to diabetes mellitus and carbohydrate metabolism. The in-depth presentation of the biochemistry, genetics, distribution, and function of the various granin proteins is not the aim of the current review, but publications on those subjects are available[1,8-14].

THE ROLE OF GRANINS IN THE SECRETION OF INSULIN

The presence of CgA[15], CgB[16-18], SgII[15] and SgVII (*VGF*, non-acronymic)[19,20] as secretory proteins has been described in animal and cellular models of pancreatic islets. Pancreatic beta cells of chromogranin A gene (*CHGA*) knockout (KO) mice were reported to have compensatory overexpression of CgB and SgII, with simultaneous insulin overproduction and fewer immature secretory granules. The CgA cleavage products betagranin (CgA_{1-128})[21], vasostatin-I (CgA_{1-76}) and catestatin (CST, $CgA_{352-372}$) are found in beta cells; pancreastatin (PST, $CgA_{250-301}$) is found in alpha cells[15,22], indicating different protein cleavage products mediated by different endoproteases [14].

Betagranin was reported to have a negative effect on glucose-stimulated insulin secretion (GSIS). Betagranin treatment of murine insulinoma cell lines was found to inhibit insulin secretion in a dose-dependent manner that was associated with dysfunction of the calcium response[21]. Normal cell function was restored when betagranin was removed. Antibodies against CgA or PST have no effect on insulin secretion, while the partial absence of CgB results in increased proinsulin synthesis [23]. Colocalization of insulin and CgB was confirmed in the *trans*-Golgi network of human and murine islet cells from healthy and insulinoma tissue[17]. Glucose-stimulated insulin, glucagon, and somatostatin secretion were decreased in chromogranin B gene (*CHGB*) KO mice in parallel with a decrease in the amount of circulating insulin and a slight decrease in renal glucose clearance. The insulin sensitivity of *CHGB* KO mice did not differ from that of wild-type mice[18]. Proinsulin processing was slowed in the absence of CgB. The density of proinsulin-containing secretory

Table 1 Names, loci, and molecular masses of granin proteins[1,2]

Name	Synonym	Locus	Number of amino acids and calculated molecular mass (kDa)
Chromogranin A	Parathyroid secretory protein 1	14q32.12	439 (49 kDa)
Chromogranin B	Secretogranin I	20pter-p12	657 (77 kDa)
Secretogranin II	Chromogranin C	2q35-q36	587 (68 kDa)
Secretogranin III	–	15q21	449 (51 kDa)
HISL-19[1]	Secretogranin IV	–	–
7B2	Secretogranin V	15q13-q14	186 (21 kDa)
NESP55	Secretogranin VI	20q13.2	201 (23 kDa)
VGF	Secretogranin VII	7q22.1	593 (65 kDa)
proSAAS	Secretogranin VIII	Xp11.23	227 (24 kDa)

[1]HISL-19 has only been confirmed with monoclonal antibodies; *in vivo* isolation has not been successful to date[2,87]. NESP55: Neuroendocrine secretory protein with an apparent molecular weight of 55,000 Daltons.

granules was altered, causing significantly slower detachment of these granules from the *trans*-Golgi network, which ultimately delayed the translocation of the granules to the plasma membrane. Although the function of cell surface receptors was not different from that of wild-type mice, the initial, rapid phase of GSIS was virtually absent in *CHGB* KO mice. The loss of rapid GSIS was compensated by increased basal insulin production, and the beta cells of *CHGB* KO mice stored and secreted twice as much proinsulin than the beta cells of wild-type mice[16,18]. These observations, seen in KO mice, are similar to the characteristics of type 2 diabetes mellitus (T2DM) in humans[18]. Stimulus-coupled insulin secretion was decreased in *VGF* (the gene that encodes SgVII protein) KO mice. An impairment of the second phase of insulin secretion was described, and secretory granules detached significantly more slowly from the *trans*-Golgi network, and was accompanied by an increase in the proinsulin level[20], similar to the effect observed in the case of CgB.

GRANIN PEPTIDES IN GLUCOSE HOMEOSTASIS

Pancreastatin

PST negatively regulates insulin sensitivity and glucose homeostasis. PST-mediated inhibition of insulin secretion promotes a high blood glucose level (hyperglycemia). Moreover, PST can: (1) Reduce the hepatic glucose uptake through inhibiting the insulin-stimulated glycogenesis in primary hepatocytes; (2) Decrease the insulin-stimulated synthesis of lipids; and (3) Regulate the expression and secretion of leptin in adipocytes, which also increases blood glucose levels[24-27]. G-protein-activated phospholipase C β3 isoforms[5,28-30] or activation of nitric oxide pathways[31-33] in hepatocytes inhibit insulin but only the former pathway has been described in adipocytes[5,30]. *CHGA* KO mice are obese, have hypertension, diminished baroreflex sensitivity, increased plasma catecholamine and adipokine levels, and lower interleukin-6 and lipid levels compared with wild-type animals[32,34]. A normal blood glucose level (euglycemia) is maintained by increased liver insulin sensitivity in *CHGA* KO mice, which is supported by the abundance of hepatic phosphoenolpyruvate carboxykinase (PEPC) and glucose-6-phosphatase (G6Pase) mRNAs. *CHGA* KO mice treated with PST are euglycemic, even in the absence of PEPC and G6Pase mRNAs[32].

The PST inhibitor peptide-8 (PSTi8)[35-39] reduces the effects of PST-induced insulin resistance. PSTi8 increases translocation of glucose transporter type 4 to the cell surface in hepatocytes and adipocytes, thereby promoting glucose uptake. It also reduces hepatic glucose release, lipid deposition, dexamethasone-induced oxidative stress; stimulates hepatocellular energy levels, and enhances the activity of glucose response protein 78[37,40]. PSTi8 treatment reduces lipogenesis, enhances fatty acid oxidation, improves glucose homeostasis *via* increased glycogenesis and glycolysis, and decreases gluconeogenesis in streptozotocin-induced diabetic mice[35,38]. The

Table 2 Cleavage products of granin proteins[1,3,88-90]

Granin protein	Cleavage product
CgA[1]	Vasostatin-I (CgA_{1-76}) and -II (CgA_{1-115})
	Betagranin (CgA_{1-128})
	CgA_{10-19} and CgA_{43-52}[1]
	Chromofungin (CgA_{47-66})
	Vasoconstriction-inhibiting factor (CgA_{79-113})
	Chromostatin ($CgA_{124-143}$)
	Chromacin ($CgA_{173-194}$)
	Pancreastatin ($CgA_{250-301}$)[1]
	WE-14 ($CgA_{324-337}$)[1]
	Cateslitin ($CgA_{344-358}$)
	Catestatin ($CgA_{352-372}$)[1]
	Parastatin ($CgA_{357-428}$)
	GE-25 ($CgA_{367-391}$)
	Serpinin ($CgA_{417-442}$)
CgB[1]	CgB_{1-41}
	GAWK ($CgB_{420-493}$)
	BAM-1745 ($CgB_{579-593}$)
	PE-11 ($CgB_{555-565}$)
	Secretolytin ($CgB_{647-657}$)
	43kDa large CgB fragment
SgII[1]	Secretoneurin (conjugate of $SgII_{133-151}$ and $SgII_{154-186}$)
	EM66 (66 amino acid long)
	Manserin ($SgII_{497-536}$)
SgIII[1]	–[2]
HISL-19	–[2]
7B2	–[2]
NESP55	LSAL ($NESP55_{159-162}$)
	GAIPIRRH ($NESP55_{234-241}$)
VGF	Neuroendocrine regulatory peptide-1 ($VGF_{281-306}$)
	Neuroendocrine regulatory peptide-2 ($VGF_{310-347}$)
	NAPP129 or VGF20 ($VGF_{417-617}$)
	TPGH ($VGF_{422-430}$)
	TLQP-21 ($VGF_{556-576}$)
	TLQP-62 or VGF10 ($VGF_{556-617}$)
	HHPD-41 ($VGF_{576-617}$)
	AQEE-11 ($VGF_{588-599}$)
	AQEE-30 ($VGF_{588-617}$)
	LQEQ-19 ($VGF_{599-617}$)
proSAAS	KEP ($proSAAS_{1-7}$)
	Big SAAS ($proSAAS_{1-26}$)
	Little SAAS ($proSAAS_{9-26}$)

	GAV
	PEN (proSAAS$_{188-209}$)
	PEN-LEN (proSAAS$_{188-227}$)
	Little LEN (proSAAS$_{212-221}$)
	Big LEN (proSAAS$_{212-227}$)

[1]Granins and their cleavage products involved in carbohydrate metabolism.
[2]No cleavage product described to date. CgA: Chromogranin A; CgB: Chromogranin B; NESP55: Neuroendocrine secretory protein with an apparent molecular weight of 55000 Daltons; SgII: Secretogranin II.

insulin-sensitizing effect of PSTi8 is equivalent to that of metformin, one of the most commonly used oral antidiabetic agents. Therefore, its potential role as a new antidiabetic agent is an ongoing area of research[39].

Catestatin

CST is indirectly associated with diabetes and carbohydrate metabolism by its effects on hypertension, obesity, and metabolic syndrome, and its possible use as a future antihypertensive or antiobesity agent has been considered[41]. External administration of CST reduces the bodyweight of obese *CHGA* KO mice[42] and can normalize catecholamine levels and baroreceptor function[34] to a state similar to that of wild-type mice. The obesity-reducing effects of CST result from enhancement of leptin receptor signaling and inhibition of alpha$_2$-adrenergic receptor signaling[43]. *CHGA* KO mice fed a high-fat diet have elevated insulin levels. Treatment with external CST normalizes the glucose metabolism of hepatocytes and improves the insulin sensitivity of the animals[44]. Obese children and adolescents have a significantly lower serum CST levels than those in healthy controls. In a cohort of obese children, those with any symptoms of metabolic syndrome or increased cardiovascular risk had the lowest serum CST levels[45].

ROLES OF GRANINS IN DIABETES MELLITUS

Diabetes mellitus is one of the most prevalent diseases in our time. Recent estimates of the prevalence range from 4% to 10%, and there are more than 460 million diabetes patients worldwide. Approximately 10% of diabetes patients have type 1 diabetes mellitus (T1DM); most of the remaining patients have T2DM[46]. The former has an autoimmune pathomechanism; the latter is a consequence of insulin resistance. Furthermore, T1DM develops mostly in younger people, while T2DM develops at later ages[47,48]. Although our knowledge on the pathomechanism of diabetes is very extensive, new relationships between diabetes and molecules involved in the development or subsequent progression of the disease is still a recent and popular area of research[49]. Examples of these recently described molecules include CgA, CgB, SgII, and secretogranin III (SgIII), the CgA cleavage peptide derivatives PST, WE-14, and small N-terminal fragments CgA$_{10-19}$ and CgA$_{43-52}$.

Chromogranin A

CgA in T1DM

The role of CgA in the development of T1DM has been demonstrated by the absence of T1DM in *CHGA* KO nonobese diabetic (NOD) mice, in contrast to wild-type NOD mice (a TIDM animal model system)[50]. Furthermore, insulitis, the inflammation of the pancreatic islets, occurred in only one-fifth of *CHGA* KO NOD mice, but did occur in all wild-type NOD mice. Insulitis was accompanied by significantly decreased numbers of infiltrating CD4$^+$ and CD8$^+$ T cells in *CHGA* KO NOD mice. It should be noted that it was not possible to investigate more accurately whether the absence of the entire CgA molecule or any of its cleavage products prevented the development of T1DM in the *CHGA* KO NOD mouse model.

CgA has been reported to be elevated in approximately 20% of patients with T1DM when examined many years after the onset of the disease[51]. An even greater

prevalence of high CgA levels was found in another study[52]. A positive correlation has been found between serum CgA and glycated hemoglobin (HbA_{1C}) levels, with a slight but steady elevation of CgA with the increased duration of T1DM, indicating that CgA does not only contribute to T1DM pathogenesis, but also to disease progression[51].

Blood CgA level is elevated in enterochromaffin-like (ECL) cell hyperplasia, autoimmune gastritis, and in gastrointestinal neuroendocrine tumors[53,54], which are more frequent in T1DM patients than in the healthy population[53,55]. A significant proportion of patients with a high CgA level have ECL cell hyperplasia[51,55]; hence early detection of these conditions is possible with regular serum CgA level measurements[51,56]. There is a possible connection between ECL cell hyperplasia and high HbA_{1C} in T1DM patients with high CgA. It is known from animal experiments that 70%-90% of the circulating PST is produced by gastric ECL cells[57], and PST is actively involved in the regulation of glucose homeostasis[5]. The worsened metabolic status and high CgA levels may result from the hyperplasia of ECL cells, which can be further impaired by the appearance of more advanced clinical symptoms and comorbidities.

CgA cleavage products in T1DM

The CgA cleavage products WE-14 ($CgA_{324-337}$)[7], CgA_{10-19}, and CgA_{43-52}[58] are newly discovered autoantigens involved in the pathogenesis of T1DM. Embryonic medullary thymic epithelial cells do not contain CgA mRNA, which may serve as a cause for the insufficient deletion of CgA-reactive T cells[7,59] and autoimmunity against CgA-producing pancreatic beta cells. Among the aforementioned peptide products, CgA_{10-19}, and CgA_{43-52} induced $CD8^+$ T cell proliferation and displayed increased cytotoxic activity in both human T1DM patients and NOD mice[58]. In contrast, WE-14 has been shown to have $CD4^+$ T cell autoreactivity[7] that does not occur in other gastro-entero-pancreatic tissues, except for pancreatic beta cells[60]. WE-14 presumably interacts with the major histocompatibility complex (MHC) class II antigens outside of the normal peptide binding grooves of MHC molecules, as WE-14 lacks the N-terminal amino acids that easily bind to the MHC class II antigen-binding sites[7]. The above observation that the antigenicity of WE-14 occurs only in pancreatic islets is presumably depends on a difference in the proteolytic processing of CgA in beta cells [7].

The modification of WE-14 by enzyme tissue transglutaminase (TGase)[61,62] or *in vitro* N-terminal arginine-leucine-glycine-leucine amino acid addition[63] dramatically increases its antigenic activity. Covalent cross-linking[14] between the side chains of glutamine and lysine caused by TGase[64] treatment increases the antigenicity of WE-14[65]. Similar to animal models, newly diagnosed T1DM patients have also been shown to exhibit elevated WE-14 antigenicity[62]. Antigenicity can be further increased if the patient's blood has been treated with TGase *in vitro*[62].

Hybrid insulin peptides (HIPs) are formed by the coupling of proinsulin and other peptides, are stored within the same secretory granules[66], and include a peptide called 2.5HIP, which is formed by a fusion of a C-peptide fragment and WE-14[67]. $CD4^+$ T cell autoimmunity against 2.5HIP was demonstrated in NOD mice[66,67]. Peripheral NOD mouse-specific CgA-reactive T cells (BDC2.5) can bind 2.5HIP with up to 100 times higher affinity than WE-14 or CgA_{29-42} alone[68], and the number of these HIP-reactive T cells increases with disease progression[66,67]. Human HIP-reactive $CD4^+$ T cells have also been identified[66]. The development of T1DM can be prevented for more than 2 mo by transferring preactivated BDC2.5 T cells and 2.5HIP nanoparticles into NOD mice, whereas the disease manifested in untreated mice within 10 d[69].

Treating young NOD mice with liposomes containing a CgA mimotope (amino acid chain: AHHPIWARMDA) and the immunomodulator calcitriol (1α,25-dihydroxyvitamin D3) can postpone the development of T1DM[70]. Furthermore, the adoptive transfer of $CD4^+$ T cells from liposome-treated animals into NOD severe-combined-immunodeficiency mice also suppressed the development of the disease[70].

CgA and its cleavage product PST in other forms of diabetes

The few published data on the relationship between CgA and T2DM are somewhat controversial. Kogawa *et al*[71,72] reported that salivary and serum CgA were significantly higher in T2DM patients than in healthy controls and patients with higher CgA values had worse glycemic control ($HbA_{1C} \geq 7.0\%$)[71]. Impaired salivary flow was correlated with increased serum and salivary CgA levels and was associated with two genetic variants of *CHGA* (rs9658635 and rs9658655)[72]. In contrast to those findings, another study found that an almost negligible portion of T2DM patients had

serum CgA levels above the normal upper limit (> 98.1 ng/mL)[73], and no differences were found in the laboratory results and anamnestic data between the groups with normal or high serum CgA levels[73].

Postprandial serum PST levels are significantly higher in patients with prediabetes [74] or T2DM[75] compared with healthy controls, and are associated with consequent hyperglycemia[75], possibly because of the effect of PST on GSIS[75,76]. Fasting PST levels of the patients and controls did not differ[75]. Another study found that obese T2DM patients had significantly higher PST levels than obese and healthy nonobese control subjects, and that weight loss did not affect the differences in PST levels[77]. Serum PST is increased in patients with gestational diabetes, and positive correlations of PST, epinephrine, and norepinephrine levels have also been observed[78].

CHROMOGRANIN B AND SECRETOGRANINS

Even though a few hundred publications on CgB are available, very little is known about its relationship to diabetes. CgB has been reported to play a role in physiological insulin secretion[16-18] and its posttranslational changes[79], altered processing[80], and decreased serum values[81] that have been observed in human diabetes. The expression of *CHGB* in the pancreatic islets was lower in human T2DM patients compared with healthy subjects[79]. T2DM patients treated with intensive conservative insulin treatment had a significantly (approximately 20%) lower CgB level than T2DM patients treated with other regimens of antidiabetic drugs, or healthy controls. The serum CgB levels in T1DM were approximately 80% of the levels in control subjects, suggesting that pancreatic beta cells may produce a significant amount of circulating CgB. Furthermore, an assumption has been made that diabetes heavily affects CgB production. The autoimmune destruction of pancreatic beta cells in T1DM, and the more advanced state of the disease in T2DM, which is usually also associated with beta cell impairment, could cause the lower CgB levels. However, further studies are needed to test that hypothesis[81].

Diabetic retinopathy (DR), in which choroidal and retinal microvascular changes occur as complications of diabetes mellitus[82], can be characterized by an altered processing of granins in the vitreous[80]. Small peptide fragments of CgA, CgB, and SgII, which have been proposed to have anti-inflammatory properties, are rare in the vitreous of DR patients, but large fragments are rare in healthy subjects. Some authors have raised the possibility that the absence of small granin fragments may play a role in the pathogenesis of DR: Posttranslational processing of granins may be damaged because of some diabetes-specific reasons that ultimately lead to the impairment of the intraocular angiogenic balance, thus contributing to the neovascularization[80].

SgIII is a recently discovered DR-associated ligand with pro-angiogenic activity and selective binding. Based on cellular and animal-model studies, the effects of SgIII are restricted to the pathological condition, suggesting that the antibody against SgIII might be useful as a selective, anti-angiogenic drug in DR[83]. The angiogenic effect of SgIII could have been blocked *via* inhibition of the mitogen-activated protein kinase and extracellular signal-regulated kinase signaling pathways[84]. Consistent with the findings of animal studies, SgIII has been found only in the vitreous in humans[85]. Increased SgIII levels has been found in DR patients compared with retinopathy originating in patients without diabetes. Moreover, high lipid levels and a high body mass index, which are characteristic of T2DM[48] have been described as risk factors of DR[86], and have been associated with even higher SgIII levels[85].

CONCLUSION

Granin proteins are produced by various neuronal, neuroendocrine, and endocrine cell types of different organs throughout the body. They contribute intracellularly to the selective secretion of various peptides. A variety of extracellular functions of biologically active cleavage products have also emerged during their evolution. Recent studies have reported that CgA, CgB, SgII, SgIII, SgVII and some of the CgA cleavage products influence glucose homeostasis and different forms of diabetes mellitus. CgA and its peptide derivatives take part in the development and subsequent progression of T1DM, and also regulate glucose homeostasis. CgB and SgVII are prominent in physiological insulin secretion, and SgII and SgIII mainly contribute to DR. More data on the activity of granins is available for T1DM than for T2DM. The potential application of PSTi8, CST, and antibodies against SgIII as future medications further

increases the importance of granins in diabetes. Although our understanding of granin proteins in relation to glucose homeostasis and diabetes mellitus continuously extends, the most recent studies pose new challenges and raise more questions than they answer. To properly answer these questions, further clinical and experimental studies are needed.

ACKNOWLEDGEMENTS

We are grateful to Mark Eyre for English proofreading.

REFERENCES

1 **Bartolomucci A**, Possenti R, Mahata SK, Fischer-Colbrie R, Loh YP, Salton SR. The extended granin family: structure, function, and biomedical implications. *Endocr Rev* 2011; **32**: 755-797 [PMID: 21862681 DOI: 10.1210/er.2010-0027]

2 **Feldman SA**, Eiden LE. The chromogranins: their roles in secretion from neuroendocrine cells and as markers for neuroendocrine neoplasia. *Endocr Pathol* 2003; **14**: 3-23 [PMID: 12746559 DOI: 10.1385/ep:14:1:3]

3 **Helle KB**, Metz-Boutigue MH, Cerra MC, Angelone T. Chromogranins: from discovery to current times. *Pflugers Arch* 2018; **470**: 143-154 [PMID: 28875377 DOI: 10.1007/s00424-017-2027-6]

4 **Simon JP**, Aunis D. Biochemistry of the chromogranin A protein family. *Biochem J* 1989; **262**: 1-13 [PMID: 2684154 DOI: 10.1042/bj2620001]

5 **Sánchez-Margalet V**, González-Yanes C, Najib S, Santos-Alvarez J. Metabolic effects and mechanism of action of the chromogranin A-derived peptide pancreastatin. *Regul Pept* 2010; **161**: 8-14 [PMID: 20184923 DOI: 10.1016/j.regpep.2010.02.005]

6 **Strub JM**, Goumon Y, Lugardon K, Capon C, Lopez M, Moniatte M, Van Dorsselaer A, Aunis D, Metz-Boutigue MH. Antibacterial activity of glycosylated and phosphorylated chromogranin A-derived peptide 173-194 from bovine adrenal medullary chromaffin granules. *J Biol Chem* 1996; **271**: 28533-28540 [PMID: 8910482 DOI: 10.1074/jbc.271.45.28533]

7 **Stadinski BD**, Delong T, Reisdorph N, Reisdorph R, Powell RL, Armstrong M, Piganelli JD, Barbour G, Bradley B, Crawford F, Marrack P, Mahata SK, Kappler JW, Haskins K. Chromogranin A is an autoantigen in type 1 diabetes. *Nat Immunol* 2010; **11**: 225-231 [PMID: 20139986 DOI: 10.1038/ni.1844]

8 **Fischer-Colbrie R**, Eder S, Lovisetti-Scamihorn P, Becker A, Laslop A. Neuroendocrine secretory protein 55: a novel marker for the constitutive secretory pathway. *Ann N Y Acad Sci* 2002; **971**: 317-322 [PMID: 12438142 DOI: 10.1111/j.1749-6632.2002.tb04486.x]

9 **Fischer-Colbrie R**, Laslop A, Kirchmair R. Secretogranin II: molecular properties, regulation of biosynthesis and processing to the neuropeptide secretoneurin. *Prog Neurobiol* 1995; **46**: 49-70 [PMID: 7568909 DOI: 10.1016/0301-0082(94)00060-u]

10 **Hendy GN**, Bevan S, Mattei MG, Mouland AJ. Chromogranin A. *Clin Invest Med* 1995; **18**: 47-65 [PMID: 7768066]

11 **Winkler H**, Fischer-Colbrie R. The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* 1992; **49**: 497-528 [PMID: 1501763 DOI: 10.1016/0306-4522(92)90222-n]

12 **Li W**, Webster KA, LeBlanc ME, Tian H. Secretogranin III: a diabetic retinopathy-selective angiogenic factor. *Cell Mol Life Sci* 2018; **75**: 635-647 [PMID: 28856381 DOI: 10.1007/s00018-017-2635-5]

13 **Mbikay M**, Seidah NG, Chrétien M. Neuroendocrine secretory protein 7B2: structure, expression and functions. *Biochem J* 2001; **357**: 329-342 [PMID: 11439082 DOI: 10.1042/0264-6021:3570329]

14 **Herold Z**, Doleschall M, Kovesdi A, Patocs A, Somogyi A. Chromogranin A and its role in the pathogenesis of diabetes mellitus. *Endokrynol Pol* 2018; **69**: 598-610 [PMID: 30074235 DOI: 10.5603/EP.a2018.0052]

15 **Wollam J**, Mahata S, Riopel M, Hernandez-Carretero A, Biswas A, Bandyopadhyay GK, Chi NW, Eiden LE, Mahapatra NR, Corti A, Webster NJG, Mahata SK. Chromogranin A regulates vesicle storage and mitochondrial dynamics to influence insulin secretion. *Cell Tissue Res* 2017; **368**: 487-501 [PMID: 28220294 DOI: 10.1007/s00441-017-2580-5]

16 **Bearrows SC**, Bauchle CJ, Becker M, Haldeman JM, Swaminathan S, Stephens SB. Chromogranin B regulates early-stage insulin granule trafficking from the Golgi in pancreatic islet β-cells. *J Cell Sci* 2019; **132** [PMID: 31182646 DOI: 10.1242/jcs.231373]

17 **Giordano T**, Brigatti C, Podini P, Bonifacio E, Meldolesi J, Malosio ML. Beta cell chromogranin B is partially segregated in distinct granules and can be released separately from insulin in response to stimulation. *Diabetologia* 2008; **51**: 997-1007 [PMID: 18437352 DOI: 10.1007/s00125-008-0980-5]

18 **Obermüller S**, Calegari F, King A, Lindqvist A, Lundquist I, Salehi A, Francolini M, Rosa P, Rorsman P, Huttner WB, Barg S. Defective secretion of islet hormones in chromogranin-B deficient mice. *PLoS One* 2010; **5**: e8936 [PMID: 20126668 DOI: 10.1371/journal.pone.0008936]

19 **Cocco C**, Brancia C, Pirisi I, D'Amato F, Noli B, Possenti R, Ferri GL. VGF metabolic-related gene: distribution of its derived peptides in mammalian pancreatic islets. *J Histochem Cytochem* 2007; **55**: 619-628 [PMID: 17312015 DOI: 10.1369/jhc.6A7040.2007]

20 **Stephens SB**, Edwards RJ, Sadahiro M, Lin WJ, Jiang C, Salton SR, Newgard CB. The Prohormone VGF Regulates β Cell Function *via* Insulin Secretory Granule Biogenesis. *Cell Rep* 2017; **20**: 2480-2489 [PMID: 28877479 DOI: 10.1016/j.celrep.2017.08.050]

21 **Schmid GM**, Meda P, Caille D, Wargent E, O'Dowd J, Hochstrasser DF, Cawthorne MA, Sanchez JC. Inhibition of insulin secretion by betagranin, an N-terminal chromogranin A fragment. *J Biol Chem* 2007; **282**: 12717-12724 [PMID: 17289672 DOI: 10.1074/jbc.M700788200]

22 **Cetin Y**, Aunis D, Bader MF, Galindo E, Jörns A, Bargsten G, Grube D. Chromostatin, a chromogranin A-derived bioactive peptide, is present in human pancreatic insulin (beta) cells. *Proc Natl Acad Sci USA* 1993; **90**: 2360-2364 [PMID: 8096340 DOI: 10.1073/pnas.90.6.2360]

23 **Karlsson E**, Stridsberg M, Sandler S. Chromogranin-B regulation of IAPP and insulin secretion. *Regul Pept* 2000; **87**: 33-39 [PMID: 10710286 DOI: 10.1016/s0167-0115(99)00105-6]

24 **González-Yanes C**, Sánchez-Margalet V. Pancreastatin, a chromogranin A-derived peptide, inhibits leptin and enhances UCP-2 expression in isolated rat adipocytes. *Cell Mol Life Sci* 2003; **60**: 2749-2756 [PMID: 14685697 DOI: 10.1007/s00018-003-3346-7]

25 **Sánchez V**, Lucas M, Calvo JR, Goberna R. Glycogenolytic effect of pancreastatin in isolated rat hepatocytes is mediated by a cyclic-AMP-independent Ca(2+)-dependent mechanism. *Biochem J* 1992; **284** (Pt 3): 659-662 [PMID: 1377910 DOI: 10.1042/bj2840659]

26 **Schmidt WE**, Creutzfeldt W. Pancreastatin--a novel regulatory peptide? *Acta Oncol* 1991; **30**: 441-449 [PMID: 1854501 DOI: 10.3109/02841869109092399]

27 **Tatemoto K**, Efendić S, Mutt V, Makk G, Feistner GJ, Barchas JD. Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* 1986; **324**: 476-478 [PMID: 3537810 DOI: 10.1038/324476a0]

28 **Ahrén B**, Bertrand G, Roye M, Ribes G. Pancreastatin modulates glucose-stimulated insulin secretion from the perfused rat pancreas. *Acta Physiol Scand* 1996; **158**: 63-70 [PMID: 8876749 DOI: 10.1046/j.1365-201X.1996.525291000.x]

29 **Sanchez V**, Calvo JR, Goberna R. Glycogenolytic effect of pancreastatin in the rat. *Biosci Rep* 1990; **10**: 87-91 [PMID: 2187544 DOI: 10.1007/BF01116856]

30 **Valicherla GR**, Hossain Z, Mahata SK, Gayen JR. Pancreastatin is an endogenous peptide that regulates glucose homeostasis. *Physiol Genomics* 2013; **45**: 1060-1071 [PMID: 24064537 DOI: 10.1152/physiolgenomics.00131.2013]

31 **Sánchez-Margalet V**, González-Yanes C, Najib S. Pancreastatin, a chromogranin A-derived peptide, inhibits DNA and protein synthesis by producing nitric oxide in HTC rat hepatoma cells. *J Hepatol* 2001; **35**: 80-85 [PMID: 11495046 DOI: 10.1016/s0168-8278(01)00071-x]

32 **Gayen JR**, Saberi M, Schenk S, Biswas N, Vaingankar SM, Cheung WW, Najjar SM, O'Connor DT, Bandyopadhyay G, Mahata SK. A novel pathway of insulin sensitivity in chromogranin A null mice: a crucial role for pancreastatin in glucose homeostasis. *J Biol Chem* 2009; **284**: 28498-28509 [PMID: 19706599 DOI: 10.1074/jbc.M109.020636]

33 **Mosén H**, Salehi A, Henningsson R, Lundquist I. Nitric oxide inhibits, and carbon monoxide activates, islet acid alpha-glucoside hydrolase activities in parallel with glucose-stimulated insulin secretion. *J Endocrinol* 2006; **190**: 681-693 [PMID: 17003269 DOI: 10.1677/joe.1.06890]

34 **Gayen JR**, Gu Y, O'Connor DT, Mahata SK. Global disturbances in autonomic function yield cardiovascular instability and hypertension in the chromogranin a null mouse. *Endocrinology* 2009; **150**: 5027-5035 [PMID: 19819970 DOI: 10.1210/en.2009-0429]

35 **Gupta AP**, Garg R, Singh P, Goand UK, Syed AA, Valicherla GR, Riyazuddin M, Mugale MN, Gayen JR. Pancreastatin inhibitor PSTi8 protects the obesity associated skeletal muscle insulin resistance in diet induced streptozotocin-treated diabetic mice. *Eur J Pharmacol* 2020; **881**: 173204 [PMID: 32439261 DOI: 10.1016/j.ejphar.2020.173204]

36 **Gupta AP**, Syed AA, Garg R, Goand UK, Singh P, Riyazuddin M, Valicherla GR, Husain A, Gayen JR. Pancreastatin inhibitor PSTi8 attenuates hyperinsulinemia induced obesity and inflammation mediated insulin resistance *via* MAPK/NOX3-JNK pathway. *Eur J Pharmacol* 2019; **864**: 172723 [PMID: 31586632 DOI: 10.1016/j.ejphar.2019.172723]

37 **Hossain Z**, Valicherla GR, Gupta AP, Syed AA, Riyazuddin M, Chandra S, Siddiqi MI, Gayen JR. Discovery of pancreastatin inhibitor PSTi8 for the treatment of insulin resistance and diabetes: studies in rodent models of diabetes mellitus. *Sci Rep* 2018; **8**: 8715 [PMID: 29880906 DOI: 10.1038/s41598-018-27018-8]

38 **Valicherla GR**, Gupta AP, Hossain Z, Riyazuddin M, Syed AA, Husain A, Lahiri S, Dave KM, Gayen JR. Pancreastatin inhibitor, PSTi8 ameliorates metabolic health by modulating AKT/GSK-3β and PKCλ/ζ/SREBP1c pathways in high fat diet induced insulin resistance in peri-/post-menopausal rats. *Peptides* 2019; **120**: 170147 [PMID: 31473204 DOI: 10.1016/j.peptides.2019.170147]

39 **Valicherla GR**, Riyazuddin M, Shahi S, Gupta AP, Syed AA, Husain A, Gayen JR. LC-ESI-MS/MS assay development and validation of a novel antidiabetic peptide PSTi8 in mice plasma using SPE: An application to pharmacokinetics. *J Pharm Biomed Anal* 2020; **180**: 113074 [PMID: 31891874 DOI: 10.1016/j.jpba.2019.113074]

40 **Gupta AP**, Singh P, Garg R, Valicherla GR, Riyazuddin M, Syed AA, Hossain Z, Gayen JR. Pancreastatin inhibitor activates AMPK pathway *via* GRP78 and ameliorates dexamethasone induced fatty liver disease in C57BL/6 mice. *Biomed Pharmacother* 2019; **116**: 108959 [PMID: 31108350

DOI: 10.1016/j.biopha.2019.108959]
41 **Bandyopadhyay GK**, Mahata SK. Chromogranin A Regulation of Obesity and Peripheral Insulin Sensitivity. *Front Endocrinol (Lausanne)* 2017; **8**: 20 [PMID: 28228748 DOI: 10.3389/fendo.2017.00020]
42 **Mahapatra NR**, O'Connor DT, Vaingankar SM, Hikim AP, Mahata M, Ray S, Staite E, Wu H, Gu Y, Dalton N, Kennedy BP, Ziegler MG, Ross J, Mahata SK. Hypertension from targeted ablation of chromogranin A can be rescued by the human ortholog. *J Clin Invest* 2005; **115**: 1942-1952 [PMID: 16007257 DOI: 10.1172/JCI24354]
43 **Kim SJ**, Tang T, Abbott M, Viscarra JA, Wang Y, Sul HS. AMPK Phosphorylates Desnutrin/ATGL and Hormone-Sensitive Lipase To Regulate Lipolysis and Fatty Acid Oxidation within Adipose Tissue. *Mol Cell Biol* 2016; **36**: 1961-1976 [PMID: 27185873 DOI: 10.1128/MCB.00244-16]
44 **Ying W**, Mahata S, Bandyopadhyay GK, Zhou Z, Wollam J, Vu J, Mayoral R, Chi NW, Webster NJG, Corti A, Mahata SK. Catestatin Inhibits Obesity-Induced Macrophage Infiltration and Inflammation in the Liver and Suppresses Hepatic Glucose Production, Leading to Improved Insulin Sensitivity. *Diabetes* 2018; **67**: 841-848 [PMID: 29432123 DOI: 10.2337/db17-0788]
45 **Simunovic M**, Supe-Domic D, Karin Z, Degoricija M, Paradzik M, Bozic J, Unic I, Skrabic V. Serum catestatin concentrations are decreased in obese children and adolescents. *Pediatr Diabetes* 2019; **20**: 549-555 [PMID: 30714297 DOI: 10.1111/pedi.12825]
46 **International Diabetes Federation**. IDF Diabetes Atlas. 9th ed. Brussels: International Diabetes Federation, 2019. [cited 4 January 2021]. Available from: https://diabetesatlas.org/en/
47 **Katsarou A**, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark Å. Type 1 diabetes mellitus. *Nat Rev Dis Primers* 2017; **3**: 17016 [PMID: 28358037 DOI: 10.1038/nrdp.2017.16]
48 **DeFronzo RA**, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI, Simonson DC, Testa MA, Weiss R. Type 2 diabetes mellitus. *Nat Rev Dis Primers* 2015; **1**: 15019 [PMID: 27189025 DOI: 10.1038/nrdp.2015.19]
49 **Broedbaek K**, Hilsted L. Chromogranin A as biomarker in diabetes. *Biomark Med* 2016; **10**: 1181-1189 [PMID: 27611656 DOI: 10.2217/bmm-2016-0091]
50 **Baker RL**, Bradley B, Wiles TA, Lindsay RS, Barbour G, Delong T, Friedman RS, Haskins K. Cutting Edge: Nonobese Diabetic Mice Deficient in Chromogranin A Are Protected from Autoimmune Diabetes. *J Immunol* 2016; **196**: 39-43 [PMID: 26608914 DOI: 10.4049/jimmunol.1501190]
51 **Herold Z**, Herold M, Nagy P, Patocs A, Doleschall M, Somogyi A. Serum chromogranin A level continuously rises with the progression of type 1 diabetes, and indicates the presence of both enterochromaffin-like cell hyperplasia and autoimmune gastritis. *J Diabetes Investig* 2020; **11**: 865-873 [PMID: 31883432 DOI: 10.1111/jdi.13203]
52 **Ebert A**, König J, Frommer L, Schuppan D, Kahaly GJ. Chromogranin Serves as Novel Biomarker of Endocrine and Gastric Autoimmunity. *J Clin Endocrinol Metab* 2020; **105** [PMID: 32436949 DOI: 10.1210/clinem/dgaa288]
53 **De Block CE**, De Leeuw IH, Van Gaal LF. Autoimmune gastritis in type 1 diabetes: a clinically oriented review. *J Clin Endocrinol Metab* 2008; **93**: 363-371 [PMID: 18029461 DOI: 10.1210/jc.2007-2134]
54 **Vinik AI**, Silva MP, Woltering EA, Go VL, Warner R, Caplin M. Biochemical testing for neuroendocrine tumors. *Pancreas* 2009; **38**: 876-889 [PMID: 19855234 DOI: 10.1097/MPA.0b013e3181bc0e77]
55 **De Block CE**, Colpin G, Thielemans K, Coopmans W, Bogers JJ, Pelckmans PA, Van Marck EA, Van Hoof V, Martin M, De Leeuw IH, Bouillon R, Van Gaal LF. Neuroendocrine tumor markers and enterochromaffin-like cell hyper/dysplasia in type 1 diabetes. *Diabetes Care* 2004; **27**: 1387-1393 [PMID: 15161793 DOI: 10.2337/diacare.27.6.1387]
56 **Herold Z**, Uhlyarik A, Herold M, Nagy P, Huszty GD, Rosta K, Doleschall M, Somogyi A. Regular chromogranin A monitoring facilitated the early detection of a gastrointestinal neuroendocrine tumour in a patient with type 1 diabetes. *Endokrynol Pol* 2020; **71**: 483-484 [PMID: 32856287 DOI: 10.5603/EP.a2020.0054]
57 **Kimura K**, Chen D, Lindström E, Zhao CM, Håkanson R. Evidence that rat stomach ECL cells represent the main source of circulating pancreastatin. *Regul Pept* 1997; **68**: 177-180 [PMID: 9100284 DOI: 10.1016/s0167-0115(96)02117-9]
58 **Li Y**, Zhou L, Li Y, Zhang J, Guo B, Meng G, Chen X, Zheng Q, Zhang L, Zhang M, Wang L. Identification of autoreactive CD8+ T cell responses targeting chromogranin A in humanized NOD mice and type 1 diabetes patients. *Clin Immunol* 2015; **159**: 63-71 [PMID: 25958206 DOI: 10.1016/j.clim.2015.04.017]
59 **Anderson MS**, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002; **298**: 1395-1401 [PMID: 12376594 DOI: 10.1126/science.1075958]
60 **Gleeson CM**, Curry WJ, Johnston CF, Buchanan KD. Occurrence of WE-14 and chromogranin A-derived peptides in tissues of the human and bovine gastro-entero-pancreatic system and in human neuroendocrine neoplasia. *J Endocrinol* 1996; **151**: 409-420 [PMID: 8994386 DOI: 10.1677/joe.0.1510409]
61 **Delong T**, Baker RL, He J, Barbour G, Bradley B, Haskins K. Diabetogenic T-cell clones recognize an altered peptide of chromogranin A. *Diabetes* 2012; **61**: 3239-3246 [PMID: 22912420 DOI:

10.2337/db12-0112]
62 **Gottlieb PA**, Delong T, Baker RL, Fitzgerald-Miller L, Wagner R, Cook G, Rewers MR, Michels A, Haskins K. Chromogranin A is a T cell antigen in human type 1 diabetes. *J Autoimmun* 2014; **50**: 38-41 [PMID: 24239002 DOI: 10.1016/j.jaut.2013.10.003]
63 **Jin N**, Wang Y, Crawford F, White J, Marrack P, Dai S, Kappler JW. N-terminal additions to the WE14 peptide of chromogranin A create strong autoantigen agonists in type 1 diabetes. *Proc Natl Acad Sci USA* 2015; **112**: 13318-13323 [PMID: 26453556 DOI: 10.1073/pnas.1517862112]
64 **Sollid LM**. Molecular basis of celiac disease. *Annu Rev Immunol* 2000; **18**: 53-81 [PMID: 10837052 DOI: 10.1146/annurev.immunol.18.1.53]
65 **Guillemot J**, Guérin M, Thouënnon E, Montéro-Hadjadje M, Leprince J, Lefebvre H, Klein M, Muresan M, Anouar Y, Yon L. Characterization and plasma measurement of the WE-14 peptide in patients with pheochromocytoma. *PLoS One* 2014; **9**: e88698 [PMID: 24523932 DOI: 10.1371/journal.pone.0088698]
66 **Delong T**, Wiles TA, Baker RL, Bradley B, Barbour G, Reisdorph R, Armstrong M, Powell RL, Reisdorph N, Kumar N, Elso CM, DeNicola M, Bottino R, Powers AC, Harlan DM, Kent SC, Mannering SI, Haskins K. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. *Science* 2016; **351**: 711-714 [PMID: 26912858 DOI: 10.1126/science.aad2791]
67 **Baker RL**, Jamison BL, Wiles TA, Lindsay RS, Barbour G, Bradley B, Delong T, Friedman RS, Nakayama M, Haskins K. CD4 T Cells Reactive to Hybrid Insulin Peptides Are Indicators of Disease Activity in the NOD Mouse. *Diabetes* 2018; **67**: 1836-1846 [PMID: 29976617 DOI: 10.2337/db18-0200]
68 **Liu B**, Hood JD, Kolawole EM, Woodruff DM, Vignali DA, Bettini M, Evavold BD. A Hybrid Insulin Epitope Maintains High 2D Affinity for Diabetogenic T Cells in the Periphery. *Diabetes* 2020; **69**: 381-391 [PMID: 31806623 DOI: 10.2337/db19-0399]
69 **Jamison BL**, Neef T, Goodspeed A, Bradley B, Baker RL, Miller SD, Haskins K. Nanoparticles Containing an Insulin-ChgA Hybrid Peptide Protect from Transfer of Autoimmune Diabetes by Shifting the Balance between Effector T Cells and Regulatory T Cells. *J Immunol* 2019; **203**: 48-57 [PMID: 31109955 DOI: 10.4049/jimmunol.1900127]
70 **Bergot AS**, Buckle I, Cikaluru S, Naranjo JL, Wright CM, Zheng G, Talekar M, Hamilton-Williams EE, Thomas R. Regulatory T Cells Induced by Single-Peptide Liposome Immunotherapy Suppress Islet-Specific T Cell Responses to Multiple Antigens and Protect from Autoimmune Diabetes. *J Immunol* 2020; **204**: 1787-1797 [PMID: 32111734 DOI: 10.4049/jimmunol.1901128]
71 **Kogawa EM**, Grisi DC, Falcão DP, Amorim IA, Rezende TM, da Silva IC, Silva ON, Franco OL, de Amorim RF. Impact of glycemic control on oral health status in type 2 diabetes individuals and its association with salivary and plasma levels of chromogranin A. *Arch Oral Biol* 2016; **62**: 10-19 [PMID: 26605682 DOI: 10.1016/j.archoralbio.2015.11.005]
72 **Kogawa EM**, Grisi DC, Falcão DP, Amorim IA, Rezende TM, da Silva IC, Silva ON, Franco OL, de Amorim RF. Salivary function impairment in type 2 Diabetes patients associated with concentration and genetic polymorphisms of chromogranin A. *Clin Oral Investig* 2016; **20**: 2083-2095 [PMID: 26750135 DOI: 10.1007/s00784-015-1705-z]
73 **Herold Z**, Herold M, Doleschall M, Somogyi A. [Serum chromogranin A level in type 2 diabetes patients]. *Diabetol Hung* 2020; **28**: 91-96 [DOI: 10.24121/dh.2020.9]
74 **Sánchez-Margalet V**, Valle M, Lobón JA, Maldonado A, Escobar-Jimenez F, Oliván J, Pérez-Cano R, Goberna R. Increased plasma pancreastatin-like immunoreactivity levels in non-obese patients with essential hypertension. *J Hypertens* 1995; **13**: 251-258 [PMID: 7615956]
75 **Tateishi K**, Funakoshi A, Wakasugi H, Iguchi H, Shinozaki H, Abe M, Funakoshi S, Tamamura H, Yajima H, Matsuoka Y. Plasma pancreastatin-like immunoreactivity in various diseases. *J Clin Endocrinol Metab* 1989; **69**: 1305-1308 [PMID: 2555388 DOI: 10.1210/jcem-69-6-1305]
76 **Funakoshi A**, Tateishi K, Shinozaki H, Matsumoto M, Wakasugi H. Elevated plasma levels of pancreastatin (PST) in patients with non-insulin-dependent diabetes mellitus (NIDDM). *Regul Pept* 1990; **30**: 159-164 [PMID: 2274680 DOI: 10.1016/0167-0115(90)90056-3]
77 **O'Connor DT**, Cadman PE, Smiley C, Salem RM, Rao F, Smith J, Funk SD, Mahata SK, Mahata M, Wen G, Taupenot L, Gonzalez-Yanes C, Harper KL, Henry RR, Sanchez-Margalet V. Pancreastatin: multiple actions on human intermediary metabolism in vivo, variation in disease, and naturally occurring functional genetic polymorphism. *J Clin Endocrinol Metab* 2005; **90**: 5414-5425 [PMID: 15956083 DOI: 10.1210/jc.2005-0408]
78 **Sánchez-Margalet V**, Lobón JA, González A, Fernández-Soto ML, Escobar-Jiménez F, Goberna R. Increased plasma pancreastatin-like levels in gestational diabetes: correlation with catecholamine levels. *Diabetes Care* 1998; **21**: 1951-1954 [PMID: 9802749 DOI: 10.2337/diacare.21.11.1951]
79 **Bugliani M**, Liechti R, Cheon H, Suleiman M, Marselli L, Kirkpatrick C, Filipponi F, Boggi U, Xenarios I, Syed F, Ladriere L, Wollheim C, Lee MS, Marchetti P. Microarray analysis of isolated human islet transcriptome in type 2 diabetes and the role of the ubiquitin-proteasome system in pancreatic beta cell dysfunction. *Mol Cell Endocrinol* 2013; **367**: 1-10 [PMID: 23246353 DOI: 10.1016/j.mce.2012.12.001]
80 **Fournier I**, Gaucher D, Chich JF, Bach C, Shooshtarizadeh P, Picaud S, Bourcier T, Speeg-Schatz C, Strub JM, Van Dorsselaer A, Corti A, Aunis D, Metz-Boutigue MH. Processing of chromogranins/secretogranin in patients with diabetic retinopathy. *Regul Pept* 2011; **167**: 118-124 [PMID: 21185877 DOI: 10.1016/j.regpep.2010.12.004]
81 **Herold Z**, Herold M, Rosta K, Doleschall M, Somogyi A. Lower serum chromogranin B level is

associated with type 1 diabetes and with type 2 diabetes patients with intensive conservative insulin treatment. *Diabetol Metab Syndr* 2020; **12**: 61 [PMID: 32684986 DOI: 10.1186/s13098-020-00569-5]

82 **Duh EJ**, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. *JCI Insight* 2017; **2** [PMID: 28724805 DOI: 10.1172/jci.insight.93751]

83 **LeBlanc ME**, Wang W, Chen X, Caberoy NB, Guo F, Shen C, Ji Y, Tian H, Wang H, Chen R, Li W. Secretogranin III as a disease-associated ligand for antiangiogenic therapy of diabetic retinopathy. *J Exp Med* 2017; **214**: 1029-1047 [PMID: 28330905 DOI: 10.1084/jem.20161802]

84 **Tang F**, Pacheco MTF, Chen P, Liang D, Li W. Secretogranin III promotes angiogenesis through MEK/ERK signaling pathway. *Biochem Biophys Res Commun* 2018; **495**: 781-786 [PMID: 29154827 DOI: 10.1016/j.bbrc.2017.11.080]

85 **Jiao MF**, Sun S, Wang MM, Dong LJ, Hu BJ, Liu JP, Li W, Li XR. [The observation of secretogranin III in the peripheral blood and vitreous of patients with diabetic retinopathy]. *Zhonghua Yan Ke Za Zhi* 2020; **56**: 933-937 [PMID: 33342120 DOI: 10.3760/cma.j.cn112142-20200410-00260]

86 **van Leiden HA**, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD, Polak BC. Blood pressure, lipids, and obesity are associated with retinopathy: the hoorn study. *Diabetes Care* 2002; **25**: 1320-1325 [PMID: 12145228 DOI: 10.2337/diacare.25.8.1320]

87 **Neuhold N**, Ullrich R. Secretogranin IV immunoreactivity in medullary thyroid carcinoma: an immunohistochemical study of 62 cases. *Virchows Arch A Pathol Anat Histopathol* 1993; **423**: 85-89 [PMID: 7692664 DOI: 10.1007/BF01606581]

88 **Ischia R**, Loviseti-Scamihorn P, Hogue-Angeletti R, Wolkersdorfer M, Winkler H, Fischer-Colbrie R. Molecular cloning and characterization of NESP55, a novel chromogranin-like precursor of a peptide with 5-HT1B receptor antagonist activity. *J Biol Chem* 1997; **272**: 11657-11662 [PMID: 9111083 DOI: 10.1074/jbc.272.17.11657]

89 **Bresciani E**, Possenti R, Coco S, Rizzi L, Meanti R, Molteni L, Locatelli V, Torsello A. TLQP-21, A VGF-Derived Peptide Endowed of Endocrine and Extraendocrine Properties: Focus on In Vitro Calcium Signaling. *Int J Mol Sci* 2019; **21** [PMID: 31878142 DOI: 10.3390/ijms21010130]

90 **Wardman JH**, Berezniuk I, Di S, Tasker JG, Fricker LD. ProSAAS-derived peptides are colocalized with neuropeptide Y and function as neuropeptides in the regulation of food intake. *PLoS One* 2011; **6**: e28152 [PMID: 22164236 DOI: 10.1371/journal.pone.0028152]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1093-1101

DOI: 10.4239/wjd.v12.i7.1093

ISSN 1948-9358 (online)

MINIREVIEWS

Diabetes remission after bariatric surgery

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Author contributions: Chumakova-Orin M, Vanetta C performed research and wrote the paper; Moris D and Guerron AD contributed critical revision of the manuscript for important intellectual content.

Conflict-of-interest statement: Authors have nothing to disclose.

Manuscript source: Invited manuscript

Specialty type: Surgery

Country/Territory of origin: United

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Abstract

Over the last decade, obesity rates have continued to rise in the United States as well as worldwide and are showing no signs of slowing down. This rise is in parallel with the increasing rates of type 2 diabetes mellitus (T2DM). Given the association between obesity and T2DM and their strong correlation with increased morbidity and mortality in addition to healthcare expenditure, it is important to recognize the most effective ways to combat them. Thus, we performed a review of literature that focused on assessing the outcomes of T2DM following bariatric surgery. Available evidence suggests that bariatric surgery provides better T2DM resolution in obese patients when compared to best medical management alone. Additionally, Biliopancreatic diversion with duodenal switch as well as Roux-en-Y gastric bypass have demonstrated higher rates of T2DM resolution when compared with other bariatric procedures.

Key Words: Bariatric surgery; Diabetes; Remission; Gastric Bypass; Obesity

Core Tip: Bariatric surgery is a safe and effective way to achieve diabetes remission in those with obesity *via* a variety of mechanisms, the majority of which are independent of weight loss. Available evidence suggests that bariatric surgery provides better type 2 diabetes mellitus resolution in obese patients when compared to best medical management alone.

Citation: Chumakova-Orin M, Vanetta C, Moris DP, Guerron AD. Diabetes remission after bariatric surgery. *World J Diabetes* 2021; 12(7): 1093-1101

URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1093.htm

DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1093

States

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 28, 2021
Peer-review started: January 28, 2021
First decision: May 3, 2021
Revised: May 10, 2021
Accepted: June 25, 2021
Article in press: June 25, 2021
Published online: July 15, 2021

P-Reviewer: Marchesi F
S-Editor: Liu M
L-Editor: A
P-Editor: Li JH

INTRODUCTION

Obesity rates continue to rise in the United States as well as worldwide. In fact, obesity prevalence doubled from 15% to 33% between 1980s and 2004 and was estimated to be 37.7% from 2013 to 2014[1,2]. Interestingly, some of the recent reports project that nearly 1 in 2 United States adults will have obesity [defined as body mass index (BMI) ≥ 30 kg/m^2] by 2030 and nearly 1 in 4 adults will have severe obesity (defined as BMI ≥ 35 kg/m^2) by then[3]. These numbers are alarming as obesity has been linked to developing type 2 diabetes mellitus (T2DM), coronary artery disease, non-alcoholic fatty liver disease, malignancy, amongst others, as well as lower life expectancy. Obesity was also attributable to 365000 deaths in 2000, second to only tobacco smoking [4]. Insulin resistance has been described as the main culprit for the development of T2DM in obese patients[5]. In fact, there is a > 6-fold increase in risk of developing T2DM in those with morbid obesity or BMI ≥ 40 kg/m^2[6]. Diabetes remains one of the most prevalent chronic diseases in the United States affecting about 9% of the population in 2011, with rates continuing to grow in parallel with obesity[6].

This association between obesity and diabetes has detrimental effects on morbidity and mortality and creates a significant economic burden as a result. The cost of diabetes in 2012 was reported to be 45 billion dollars, with 69 billion dollars attributed to reduced productivity and 176 billion dollars attributed to direct medical costs[7]. Additionally, the cost of diabetes is projected to reach nearly 500 dollars billion by 2030[8]. This has led to an increased interest in finding ways to successfully treat diabetes and maintain remission.

While lifestyle modifications such as diet and exercise along with pharmacotherapy can be successful in treating both obesity and T2DM, few achieve sustained weight loss and only 10% of those with T2DM achieve favorable disease control in order to minimize and prevent long-term complications[9]. This article will thus focus on reviewing the most current data on diabetes remission following bariatric surgery.

ASSOCIATION OF T2DM AND OBESITY

Obesity has been linked to the development of T2DM *via* insulin resistance. Several mechanisms have been described. One of such proposed mechanisms involves increased release of a variety of factors including non-esterified fatty acids (NEFAs), glycerol, leptin, adiponectin, proinflammatory cytokines among others from adipose tissue which in turn leads to insulin resistance[10]. This occurs *via* reduced phosphorylation of phosphatidylinositol-3-OH kinase in muscle and increased gluconeogenic enzyme expression in the liver[9]. While this leads to insulin resistance, not all obese patients will go on to develop T2DM as they are able to overcome this by increased insulin release from pancreatic β cells to correct for decreased insulin sensitivity[9]. Thus, those with β cell dysfunction are at the highest risk of developing T2DM *via* increased release of NEFAs as they not only reduce insulin sensitivity but also decrease pancreatic β cell function[9].

Standard treatment of T2DM focuses on achieving good glycemic control in order to minimize cardiovascular and other risks and is mainly achieved *via* medical management. However, this treatment modality can become challenging in patients with obesity as a variety of pharmacotherapy agents can in fact cause weight gain and thus further worsen insulin resistance[11]. This is when bariatric surgery comes into play. Though initially described as surgical treatment for weight loss, bariatric surgery has demonstrated significant effects on reducing rates of T2DM in addition to improving cardiovascular health and thus reducing morbidity and mortality[12]. These beneficial effects are achieved *via* a multitude of mechanisms beyond weight loss.

MECHANISMS OF T2DM REMISSION FOLLOWING BARIATRIC SURGERY

Weight loss

While the exact mechanism for T2DM remission following bariatric surgery is not fully understood, several have been proposed. One such mechanism involves reduced caloric intake which in turn leads to significant weight loss and subsequently improved glucose sensitivity. This was thought to be achieved *via* restrictive and/or malabsorptive properties of bariatric surgery. However, this does not explain some of

the drastic effects seen on glucose control immediately following surgery, and thus the majority of glucose-lowering is achieved prior to weight loss[13]. Additionally, scintigraphy studies demonstrate that nutrient delivery through gastric pouch is actually increased rather than restricted following Roux-en-Y gastric bypass (RYGB)[14].

Insulin sensitivity

One of the most important factors contributing to improved glucose tolerance is a significant decrease in insulin resistance fairly early following bariatric surgery. In fact, insulin resistance decreases about 50% in 1 wk following surgery and into the normal range seen in glucose tolerant patients when measured homeostatic model assessment of insulin resistance[15-17]. In addition to improved liver insulin sensitivity, insulin clearance also increases and is thought to be due to decreased caloric intake which in turn leads to decreased liver fat content. This has been demonstrated using post-operative magnetic resonance imaging of the liver following RYGB[14]. These combined effects in turn lead to decreased basal glucose concentration and are thought to improve pancreatic β cell function by decreasing the toxic effect of glucose[18].

Foregut/hindgut hypothesis

According to the foregut-hindgut hypothesis, there is an increased amount of incompletely digested food delivered to the distal intestine due to bypassed foregut. This in turn stimulates specialized L cells which facilitate the release of glucagon-like-peptide-1 (GLP-1) and peptide YY, both of which have been implicated in achieving weight loss. Additionally, both provide a favorable effect on pancreatic β cells leading to increased insulin sensitivity[18,19]. Interestingly, GLP-1 Levels rise dramatically within days of bariatric surgery stimulating pancreatic β cells. This effect of β cell stimulation is further amplified by a temporary early increase in plasma glucose levels eventually leading to increased insulin release[17]. While this hypothesis may explain the benefits seen following RYGB and biliopancreatic diversion with duodenal switch (BPD-DS), it does not explain the beneficial effects on glucose metabolism seen following sleeve gastrectomy (SG) as the intestinal tract remains in continuity[16].

Bile acids

Circulating bile acids (BA) levels also increase following bariatric surgery and are correlated with improved glucose sensitivity. This is thought to occur following reduced mixture of partially digested nutrients with BAs following surgery thus leading to higher concentration of free circulating BAs. This, in turn, leads to reduction in hepatic glucose production as well as glucogeogenesis within gut segments that are devoid of BAs[20].

METHODS

A comprehensive search of the published literature in PubMed, PubMed Central (PMC), EMBASE, Medline, and the Cochrane Register of Controlled Trials databases was conducted until January 2021. We used the guidelines of 2015 Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P). Randomised controlled trials with at least 12 mo of follow-up and prediction models of diabetes remission after bariatric surgery were included. Keywords containing "obesity", "metabolic [or] bariatric surgery", "type 2 diabetes", "diabetes remission", "predict", "prediction models" and "score" were constructed for inclusion. Only studies in english language were included.

TRIALS COMPARING T2DM REMISSION FOLLOWING BARIATRIC SURGERY

Mingrone *et al*[12] designed a randomized clinical trial (RCT) looking at 60 patients who were randomly assigned to one of the 3 groups: Conventional medical therapy, RYGB or BPD-DS. Their primary endpoint included diabetes remission which was defined as fasting glucose level < 100 mg/dL and glycosylated hemoglobin (HbA1c) < 6.5mmol/L without the use of pharmacotherapy. Fifty-six patients completed their 2 year follow up and at that time no patients in the medical group achieved remission while 75% in the RYGB and 95% in the BPD-DS were able to achieve remission (P < 0.05). Additionally, while the HbA1c levels did decrease significantly in all 3 groups

from average baseline of 8.65% ± 1.45%, the 2 surgical groups had greater degree of lowering with average numbers at 2-year follow up being the following: 7.69% ± 0.57% for the medical group, 6.35% ± 1.42% in the RYGB group and 4.95% ± 0.49% in the BPD-DS group. The authors of this study concluded that bariatric surgery was able to achieve higher rates of remission in patients with severe obesity (BMI ≥ 35 kg/m^2), and HbA1c < 6.5 mmol/L without the use of pharmacotherapy[12].

Calorie reduction or surgery: Seeking to Reduce Obesity and Diabetes Study was an RCT that assigned patients with obesity (BMI ≥ 30 kg/m^2) and T2DM to either RYGB (n = 23) or intensive lifestyle and medical management (n = 20). Patients were followed for 1 year with primary outcome measured being T2DM resolution (defined as HbA1c <6% and being off medications). During this follow up, 60% of patients following RYGB achieved remission while nearly 6% of the medical group were able to do so (P < 0.05). This study reported no life-threatening complications in the surgical group. The authors concluded that RYGB yielded higher rates of T2DM remission at 1 year when compared to medical therapy alone[21].

Surgical Treatment and Medications Potentially Eradicate Diabetes Efficiently (STAMPEDE) RCT published by Schauer *et al*[22] in 2017 provides some of the longer-term data shedding light on the effectiveness of bariatric surgery on T2DM resolution. The study randomized 150 patients with obesity and T2DM to intensive medical therapy alone *vs* medical therapy and either RYGB or SG.One hundred and thirty-four patients completed 5 year follow up with primary endpoint being achievement of HbA1c level of ≤ 6%. Of those who underwent intense medical therapy alone, 5% were able to achieve this endpoint *vs* 29% of those who also underwent RYGB (adjusted P < 0.05) and 23% of those who underwent SG (adjusted P = 0.07). In the surgical group, this endpoint was achieved without the need for hypoglycemic medications in the majority of patients, whereas none of the patients in the medical group were able to achieve that endpoint without pharmacotherapy. Additionally, 89% of patients in the surgical group were off insulin during a 5 year follow up compared to 61% of patients in the medical group[22].

Hofsø *et al*[23] designed a triple blind RCT that was conducted in Norway comparing 109 patients with morbid obesity and presence of T2DM who were randomly assigned to SG (55/109) or RYGB (54/109). The primary outcome measured during their 1 year follow up was diabetes remission defined as having HbA1c ≤ 6% while being off pharmacotherapy. 107/109 patients completed 1 year follow up demonstrating that 47% of SG patients had diabetes remission and 74% of RYGB patients had diabetes remission (P < 0.05). This study also reported 57 adverse reactions during a follow up period with 1 patient returning back to the operating room following an intra-abdominal bleed following SG, 1 patient needing blood transfusions 10 days following RYGB. No deaths were observed in this study. Thus, the authors concluded that even though pancreatic β cell function improved after both types of surgery, RYGB was found to have greater effect on T2DM resolution when compared to SG at 1 year[23].

Another recent 3-arm RCT assigned 61 patients with obesity and T2DM to one of the 3 groups: RYGB, adjustable gastric banding (AGB) or intense medical therapy and were followed for 1 year initially. After 1 year follow up, the patients were assessed for additional 4 years following introduction of lower-level lifestyle interventions. Primary endpoint of this study was T2DM remission rates (partial: Fasting plasma glucose (FPG) ≤ 125 mg/dL, HbA1c < 6.5% and off medications and complete: FPG ≤ 100 mg/dL, HbA1c < 5.7% and off medications). At 5 years, partial or complete T2DM remission was achieved in 30% of RYGB group, 19% of AGB group and 0% of medical management group (P < 0.05). Additionally, 56% of RYGB patients were off medications at 5 years as compared to 45% of AGB group and 0% of medical management group (P < 0.05). Thus, the authors concluded that their surgical management was more effective in T2DM resolution than best medical management alone[11].

More recently, a study by Mingrone *et al*[24] published their 10 year follow up results comparing metabolic surgery and medical management for T2DM at a single center in Italy. This RCT included 3 treatment arms: BPD-DS, RYGB and medical management. They had 20 patients in each arm group with 60 patients total, 57 of which completed follow up. Remission was defined as FPG ≤ 100 mg/dL, HbA1c ≤ 6.5% and being off medications. Ten-year remission rates in the intention-to-treat analysis demonstrated that 5.5% [95% confidence interval (CI) 1.0-25.7] of medical group achieved remission when compared to 50% in BPD-DS [95%CI 29.9-70.1] group and 25% in RYGB [95%CI 11.2-49.9] group (P < 0.05)[24] (Table 1).

Table 1 Bariatric surgery *vs* medical management for type 2 diabetes mellitus remission

Ref.	Pts w/ follow up/enrolled pts	Study duration, years	Medical management T2DM resolution %	RYGB T2DM resolution %	SG T2DM resolution %	BPD-DS T2DM resolution %	AGB T2DM resolution %	T2DM resolution definition	*P* value
Mingrone *et al*[12], 2012	56/60	2	0	75	N/A	95	N/A	FPG < 100 mg/dL + HbA1c < 6.5 mmol/L + no pharmacotherapy	< 0.05
Cummings *et al*[21], 2016	43/43	1	6	60	N/A	N/A	N/A	HbA1c < 6% + no pharmacotherapy	< 0.05
Schauer *et al* [22], 2017	134/150	5	5	29	23	N/A	N/A		< 0.05
Hofsø *et al* [23], 2019	107/109	1	N/A	74	47	N/A	N/A		< 0.05
Courcoulas *et al*[11], 2020	50/61	5	0	30	N/A	N/A	19		< 0.05
Mingrone *et al*[24], 2021	57/60	10	5	25	N/A	50	N/A		< 0.05

T2DM: Type 2 diabetes mellitus; RYGB: Roux-en-Y gastric bypass; SG: Sleeve gastrectomy; BPD-DS: Biliopancreatic diversion with duodenal switch; AGB: Adjustable gastric banding; FPG: Fasting plasma glucose; HbA1c: Glycocylated hemoglobin.

PREDICTORS OF DIABETES REMISSION AFTER BARIATRIC SURGERY

Several prediction models have been developed in order to assess diabetes remission following bariatric surgery. The models used are either scoring systems, in which each variable is given a specific score and the addition of scores gives the probability of diabetes remission; or a logistic regression model in which higher odds means a higher probability of diabetes remission[25]. Relevant scoring systems include the DiaRem, age, bmi, c-peptide level and duration of diabetes score (ABCD), and individualized metabolic surgery scores (IMSS).

DiaRem and DiaRem2

This model described by Still *et al*[26] in 2014 is based on a retrospective study of 690 diabetic obese patients who underwent RYGB. The preoperative factors which demonstrated to be independent predictive factors of diabetes remission were: Age, insulin use, HbA1c measurement, and type of antidiabetic medications. Preoperative insulin use was associated with the higher severity of diabetes and lower percentage of remission and was given the highest score of 10 points. The score range goes from 0 to 22, and the patients fall into one of five groups. The higher the score, the lowest the probability of diabetes remission.

Additionally, in 2019, the DiaRem2 score incorporated the duration of diabetes to the already validated DiaRem[27]. The association between "early remission" (defined as remission within the first 2 mo after surgery) and duration of diabetes, as well as early remission and score was analyzed. Patients were allocated into one of three remission groups according to their score: High (0-5), Intermediate (6-12) or Low (13-25). A highest score was associated with a decreased percentage of early remission.

ABCD

In this score proposed by Lee *et al*[28] in 2013, a first cohort of 63 patients who underwent either RYGB or mini-gastric bypass in Asia was analyzed. The four preoperative factors identified as independent risk factors for remission were: Age, baseline BMI, C-peptide level, and duration of diabetes. Patients with higher scores had higher remission rates. A modified scoring system was then tested in 510 patients, including SG patients. It showed lower remission levels after SG than those correlated to RYGB. One of the limitations of this score was that insulin and other antidiabetic medications used were not taken into account.

IMSS

IMSS was designed by Aminian *et al*[29] with the objective to guide procedure selection based on long-term diabetes remission. A sample of 659 patients who underwent RYGB or SG was analyzed. Duration of diabetes, number of diabetes medications, insulin use, and HbA1c were the four independent predictors used to develop this score. Patients were allocated into one of three stages of diabetes severity. This study included recommendations on what type of surgery to perform: RYGB for mild disease and moderate disease, and SG for severe disease. In severe diabetes, the rate of remission is lower, so the least demanding technique is preferred. Unfortunately, biliopancreatic diversion with duodenal switch was not included in this score even though this procedure has been associated with higher remissions of associated comorbidities.

Improved DiaRem model

The general limitations of the scoring systems commented is that they were based on one or two procedure types, with RYGB as the leading procedure, and they are usually limited to a population with a defined race/ethnicity. That is why Duke Group developed a logistic regression model including a diverse racial/ethnicity and a large BPD/DS sample[30]. This model was based on a retrospective review of 602 patients who underwent RYGB, SG, LAGB or BPD/DS. The objective was to analyze the relation of remission to procedure type. DiaRem score was used to assess the performance of this model in their cohort. The results showed BPD/DS patients have an approximately 229% increase in odds of having remission at 1 year compared with RYGB patients (adjusted OR 3.29; 95%CI: 1.27, 8.5).

Independent of the predictive model used, the procedure type is an independent risk factor for diabetes remission. Our results indicate BPD/DS as the procedure with a higher percentage of diabetes remission (Table 2). We believe that this procedure should be taken into account in future tools that include a recommendation of the procedure of choice.

CONCLUSION

Bariatric surgery is a safe and effective way to treat T2DM in those struggling with obesity. The effects can be seen fairly early prior to any substantial weight loss, thus highlighting the interplay of hormonal factors that leads to increased insulin sensitivity *via* activation of pancreatic β cells.

Several efforts have been made to prove the effectiveness of bariatric surgery in diabetes remission, including randomized clinical trials with 1, 3, 5 and even 10 years of follow-up. A variety of bariatric surgical procedures have demonstrated to be more effective than medical management for T2DM control, and BPD-DS and RYGB have shown some of the highest remission rates.

The literature available up to date still encounters certain limitations. For instance, in the STAMPEDE trial, patients had a relatively low BMI (mean 37 ± 3.5 kg/m^2) with 37% of them having a BMI value < 35 kg/m^2. This leaves aside the morbid and super obese population, but opens the discussion to lower the current indication guidelines to serve patients with T2DM and lower BMI.

In addition, remission was achieved in 23%-29% of patients submitted to surgery, but nothing is said about the remaining 70% who did not benefit from RYGB or SG. Could these patients benefit from another type of surgery such as biliopancreatic diversion with duodenal switch? Even the most recent information from a RCT by Mingrone *et al*[24] with the largest follow-up (10 years), exhibited maintained diabetes remission in only 37,5% of the patients when compared to conventional medical therapy.

BPD/DS is an under-utilized surgical procedure which has been associated with enhanced weight loss and resolution of comorbid disease[31], though postoperative complications are increased when compared to RYGB or SG[32]. Numerous studies, including our predictive model, have demonstrated that this procedure is related to increased weight loss and remission of diabetes and other comorbidities. Yet, its low implementation fails to show its benefits on a wide scale.

The discussion is no longer whether metabolic surgery achieves remission of diabetes or not, as this has broadly been demonstrated. Debate arises on which procedure is best for each individual patient. Predictive models are warranted to be improved once they succeed in including a large, diverse population, addressing duration of diabetes and insulin use, who are submitted to any of the surgical

Table 2 Predictors of diabetes mellitus remission following bariatric surgery

	Diarem	Diarem2	IMS	ABCD	Duke diabetes remission
Procedure	Rygb	RYGB	RYGB or SG	RYGB or mini-gastric	RYGB, SG, AGB, BPD/DS
Number of patients (*n*)	690	307	659	63	602
Variables	Insulin use	Insulin use	Insulin use	Diabetes duration	Age
					Sex
	Age	Age	Duration of diabetes	Age	Race
					Insulin use
	Hba1c	Hba1c	Hba1c	Baseline BMI	Hba1c
					BMI
	Type of antidiabetic drugs	Duration of diabetes	Number of diabetic medications	C-peptide level	Preop asthma, GERD, hypertension, hyperlipidemia, anticoagulation medication status
					Type of antidiabetic drugs
Scale	0-22 (5 groups)	0-25 (High-Intermediate-Low remission)	3 stages (Mild-Moderate-Severe)	0-10	Odds of remission according to preoperative variables and type of surgery
Recommendation on procedure of choice	No	No	Yes	No	No

RYGB: Roux-en-Y gastric bypass; SG: Sleeve gastrectomy; BPD-DS: Biliopancreatic diversion with duodenal switch; AGB: Adjustable gastric banding; HbA1c: Glycosylated hemoglobin; GERD: Gastroesophageal reflux disease.

procedures available (AGB, SG, RYGB, distal bypass, BPD-DS *etc*.).

The following steps are yet to be determined. Large multicentric studies are awaited to test and improve the existing scores, in order to ultimately develop a calculator able to predict individualized surgical outcomes. Yet, there is still a feeling of uncertainty and apprehension surrounding the fate of patients who experience diabetes relapse or weight regain after metabolic surgery.

REFERENCES

1 **Hales CM**, Fryar CD, Carroll MD, Freedman DS, Ogden CL. Trends in Obesity and Severe Obesity Prevalence in US Youth and Adults by Sex and Age, 2007-2008 to 2015-2016. *JAMA* 2018; **319**: 1723-1725 [PMID: 29570750 DOI: 10.1001/jama.2018.3060]

2 **Flegal KM**, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. *JAMA* 2016; **315**: 2284-2291 [PMID: 27272580 DOI: 10.1001/jama.2016.6458]

3 **Ward ZJ**, Bleich SN, Cradock AL, Barrett JL, Giles CM, Flax C, Long MW, Gortmaker SL. Projected U.S. State-Level Prevalence of Adult Obesity and Severe Obesity. *N Engl J Med* 2019; **381**: 2440-2450 [PMID: 31851800 DOI: 10.1056/NEJMsa1909301]

4 **Al-Sulaiti H**, Diboun I, Agha MV, Mohamed FFS, Atkin S, Dömling AS, Elrayess MA, Mazloum NA. Metabolic signature of obesity-associated insulin resistance and type 2 diabetes. *J Transl Med* 2019; **17**: 348 [PMID: 31640727 DOI: 10.1186/s12967-019-2096-8]

5 **Chobot A**, Górowska-Kowolik K, Sokołowska M, Jarosz-Chobot P. Obesity and diabetes-Not only a simple link between two epidemics. *Diabetes Metab Res Rev* 2018; **34**: e3042 [PMID: 29931823 DOI: 10.1002/dmrr.3042]

6 **Leung MY**, Carlsson NP, Colditz GA, Chang SH. The Burden of Obesity on Diabetes in the United States: Medical Expenditure Panel Survey, 2008 to 2012. *Value Health* 2017; **20**: 77-84 [PMID: 28212973 DOI: 10.1016/j.jval.2016.08.735]

7 **American Diabetes Association**. Economic costs of diabetes in the U.S. in 2012. *Diabetes Care* 2013; **36**: 1033-1046 [PMID: 23468086 DOI: 10.2337/dc12-2625]

8 **Zhang P**, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, Nichols G. Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 293-301 [PMID: 20171754 DOI: 10.1016/j.diabres.2010.01.026]

9 **Wong K**, Glovaci D, Malik S, Franklin SS, Wygant G, Iloeje U, Kan H, Wong ND. Comparison of demographic factors and cardiovascular risk factor control among U.S. adults with type 2 diabetes by

insulin treatment classification. *J Diabetes Complications* 2012; **26**: 169-174 [PMID: 22502939 DOI: 10.1016/j.jdiacomp.2012.03.006]

10 **Kahn SE**, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; **444**: 840-846 [PMID: 17167471 DOI: 10.1038/nature05482]

11 **Courcoulas AP**, Gallagher JW, Neiberg RH, Eagleton EB, DeLany JP, Lang W, Punchai S, Gourash W, Jakicic JM. Bariatric Surgery *vs* Lifestyle Intervention for Diabetes Treatment: 5-Year Outcomes From a Randomized Trial. *J Clin Endocrinol Metab* 2020; **105**: 866-876 [PMID: 31917447 DOI: 10.1210/clinem/dgaa006]

12 **Mingrone G**, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L, Nanni G, Pomp A, Castagneto M, Ghirlanda G, Rubino F. Bariatric surgery *vs* conventional medical therapy for type 2 diabetes. *N Engl J Med* 2012; **366**: 1577-1585 [PMID: 22449317 DOI: 10.1056/NEJMoa1200111]

13 **Zhu J**, Gupta R, Safwa M. The Mechanism of Metabolic Surgery: Gastric Center Hypothesis. *Obes Surg* 2016; **26**: 1639-1641 [PMID: 27075553 DOI: 10.1007/s11695-016-2175-3]

14 **Steven S**, Hollingsworth KG, Small PK, Woodcock SA, Pucci A, Aribasala B, Al-Mrabeh A, Batterham RL, Taylor R. Calorie restriction and not glucagon-like peptide-1 explains the acute improvement in glucose control after gastric bypass in Type 2 diabetes. *Diabet Med* 2016; **33**: 1723-1731 [PMID: 27589584 DOI: 10.1111/dme.13257]

15 **Jørgensen NB**, Jacobsen SH, Dirksen C, Bojsen-Møller KN, Naver L, Hvolris L, Clausen TR, Wulff BS, Worm D, Lindqvist Hansen D, Madsbad S, Holst JJ. Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with Type 2 diabetes and normal glucose tolerance. *Am J Physiol Endocrinol Metab* 2012; **303**: E122-E131 [PMID: 22535748 DOI: 10.1152/ajpendo.00073.2012]

16 **Falkén Y**, Hellström PM, Holst JJ, Näslund E. Changes in glucose homeostasis after Roux-en-Y gastric bypass surgery for obesity at day three, two months, and one year after surgery: role of gut peptides. *J Clin Endocrinol Metab* 2011; **96**: 2227-2235 [PMID: 21543426 DOI: 10.1210/jc.2010-2876]

17 **Holst JJ**, Madsbad S, Bojsen-Møller KN, Svane MS, Jørgensen NB, Dirksen C, Martinussen C. Mechanisms in bariatric surgery: Gut hormones, diabetes resolution, and weight loss. *Surg Obes Relat Dis* 2018; **14**: 708-714 [PMID: 29776493 DOI: 10.1016/j.soard.2018.03.003]

18 **Martinussen C**, Bojsen-Møller KN, Dirksen C, Jacobsen SH, Jørgensen NB, Kristiansen VB, Holst JJ, Madsbad S. Immediate enhancement of first-phase insulin secretion and unchanged glucose effectiveness in patients with type 2 diabetes after Roux-en-Y gastric bypass. *Am J Physiol Endocrinol Metab* 2015; **308**: E535-E544 [PMID: 25628424 DOI: 10.1152/ajpendo.00506.2014]

19 **Makris MC**, Alexandrou A, Papatsoutsos EG, Malietzis G, Tsilimigras DI, Guerron AD, Moris D. Ghrelin and Obesity: Identifying Gaps and Dispelling Myths. A Reappraisal. *In Vivo* 2017; **31**: 1047-1050 [PMID: 29102924 DOI: 10.21873/invivo.11168]

20 **Batterham RL**, Cummings DE. Mechanisms of Diabetes Improvement Following Bariatric/Metabolic Surgery. *Diabetes Care* 2016; **39**: 893-901 [PMID: 27222547 DOI: 10.2337/dc16-0145]

21 **Cummings DE**, Arterburn DE, Westbrook EO, Kuzma JN, Stewart SD, Chan CP, Bock SN, Landers JT, Kratz M, Foster-Schubert KE, Flum DR. Gastric bypass surgery *vs* intensive lifestyle and medical intervention for type 2 diabetes: the CROSSROADS randomised controlled trial. *Diabetologia* 2016; **59**: 945-953 [PMID: 26983924 DOI: 10.1007/s00125-016-3903-x]

22 **Schauer PR**, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, Navaneethan SD, Singh RP, Pothier CE, Nissen SE, Kashyap SR; STAMPEDE Investigators. Bariatric Surgery *vs* Intensive Medical Therapy for Diabetes - 5-Year Outcomes. *N Engl J Med* 2017; **376**: 641-651 [PMID: 28199805 DOI: 10.1056/NEJMoa1600869]

23 **Hofsø D**, Fatima F, Borgeraas H, Birkeland KI, Gulseth HL, Hertel JK, Johnson LK, Lindberg M, Nordstrand N, Cvancarova Småstuen M, Stefanovski D, Svanevik M, Gretland Valderhaug T, Sandbu R, Hjelmesæth J. Gastric bypass *vs* sleeve gastrectomy in patients with type 2 diabetes (Oseberg): a single-centre, triple-blind, randomised controlled trial. *Lancet Diabetes Endocrinol* 2019; **7**: 912-924 [PMID: 31678062 DOI: 10.1016/S2213-8587(19)30344-4]

24 **Mingrone G**, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Capristo E, Chamseddine G, Bornstein SR, Rubino F. Metabolic surgery *vs* conventional medical therapy in patients with type 2 diabetes: 10-year follow-up of an open-label, single-centre, randomised controlled trial. *Lancet* 2021; **397**: 293-304 [PMID: 33485454 DOI: 10.1016/S0140-6736(20)32649-0]

25 **Shen SC**, Wang W, Tam KW, Chen HA, Lin YK, Wang SY, Huang MT, Su YH. Validating Risk Prediction Models of Diabetes Remission After Sleeve Gastrectomy. *Obes Surg* 2019; **29**: 221-229 [PMID: 30251094 DOI: 10.1007/s11695-018-3510-7]

26 **Still CD**, Wood GC, Benotti P, Petrick AT, Gabrielsen J, Strodel WE, Ibele A, Seiler J, Irving BA, Celaya MP, Blackstone R, Gerhard GS, Argyropoulos G. Preoperative prediction of type 2 diabetes remission after Roux-en-Y gastric bypass surgery: a retrospective cohort study. *Lancet Diabetes Endocrinol* 2014; **2**: 38-45 [PMID: 24579062 DOI: 10.1016/S2213-8587(13)70070-6]

27 **Still CD**, Benotti P, Mirshahi T, Cook A, Wood GC. DiaRem^2: Incorporating duration of diabetes to improve prediction of diabetes remission after metabolic surgery. *Surg Obes Relat Dis* 2019; **15**: 717-724 [PMID: 30686670 DOI: 10.1016/j.soard.2018.12.020]

28 **Lee WJ**, Hur KY, Lakadawala M, Kasama K, Wong SK, Chen SC, Lee YC, Ser KH. Predicting success of metabolic surgery: age, body mass index, C-peptide, and duration score. *Surg Obes Relat Dis* 2013; **9**: 379-384 [PMID: 22963817 DOI: 10.1016/j.soard.2012.07.015]

29 **Aminian A**, Brethauer SA, Andalib A, Nowacki AS, Jimenez A, Corcelles R, Hanipah ZN, Punchai S, Bhatt DL, Kashyap SR, Burguera B, Lacy AM, Vidal J, Schauer PR. Individualized Metabolic Surgery Score: Procedure Selection Based on Diabetes Severity. *Ann Surg* 2017; **266**: 650-657 [PMID: 28742680 DOI: 10.1097/SLA.0000000000002407]

30 **Guerron AD**, Perez JE, Risoli T Jr, Lee HJ, Portenier D, Corsino L. Performance and improvement of the DiaRem score in diabetes remission prediction: a study with diverse procedure types. *Surg Obes Relat Dis* 2020; **16**: 1531-1542 [PMID: 32690456 DOI: 10.1016/j.soard.2020.05.010]

31 **Dorman RB**, Rasmus NF, al-Haddad BJ, Serrot FJ, Slusarek BM, Sampson BK, Buchwald H, Leslie DB, Ikramuddin S. Benefits and complications of the duodenal switch/biliopancreatic diversion compared to the Roux-en-Y gastric bypass. *Surgery* 2012; **152**: 758-65 [PMID: 22959653 DOI: 10.1016/j.surg.2012.07.023]

32 **Anderson B**, Gill RS, de Gara CJ, Karmali S, Gagner M. Biliopancreatic diversion: the effectiveness of duodenal switch and its limitations. *Gastroenterol Res Pract* 2013; **2013**: 974762 [PMID: 24639868 DOI: 10.1155/2013/974762]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1102-1115

DOI: 10.4239/wjd.v12.i7.1102

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Basic Study

Decarboxylated osteocalcin, a possible drug for type 2 diabetes, triggers glucose uptake in MG63 cells

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Author contributions: Jin S designed the study, performed the experiments and data analysis, the obtained the funding, and contributed to paper writing; Chang XC and Ao N contributed to obtaining the funding and performed the experiments; Wen J and Yang J contributed to paper writing; Zhang KY and Suo LN performed the data analysis; Du J supervised the project, reviewed and edited the manuscript, and managed the submission process.

Supported by Provincial Science and Technology Department Natural Fund Guidance Project, No. 2019-ZD-0774; National Natural Science Foundation of China, No. 81470998; Liaoning Ministry of Education, No. LQNK201715; and Liaoning Provincial Doctor Start up Fund, No. 20180540008.

Institutional review board statement: Because the objects of this study were cells, not the

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Abstract

BACKGROUND

Uncarboxylated osteocalcin (GluOC) has been reported to improve glucose metabolism, prevent type 2 diabetes, and decrease the severity of obesity in mice with type 2 diabetes. GluOC can increase glucose uptake in a variety of cells. Glucose metabolism is the main source of energy for osteoblast proliferation and differentiation. We hypothesized that decarboxylated osteocalcin (dcOC), a kind of GluOC, can increase glucose uptake in MG63 cells (osteoblast-like osteosarcoma cells) and influence their proliferation and differentiation.

AIM

To investigate the effects of dcOC on glucose uptake in human osteoblast-like osteosarcoma cells and the possible signaling pathways involved.

METHODS

MG63 cells (human osteoblast-like osteosarcoma cells) were treated with dcOC (0, 0.3, 3, 10, or 30 ng/mL) for 1 and 72 h, and glucose uptake was measured by flow cytometry. The effect of dcOC on cell proliferation was measured with a CCK-8 assay, and alkaline phosphatase (ALP) enzyme activity was measured. PI3K was inhibited with LY294002, and hypoxia-inducible factor 1 alpha (HIF-1α) was silenced with siRNA. Then, GPRC6A (G protein-coupled receptor family C group 6 subtype A), total Akt, phosphorylated Akt, HIF-1α, and glucose transporter 1 (GLUT1) levels were measured by Western blot to elucidate the possible pathways by which dcOC modulates glucose uptake.

RESULTS

The glucose uptake of MG63 cells was significantly increased compared with that

of the paired control cells after short-term (1 h) treatment with dcOC at different concentrations (0.3, 3, and 10 ng/mL groups, $P < 0.01$; 30 ng/mL group, $P < 0.05$). Glucose uptake of MG63 cells was significantly increased compared with that of the paired control cells after long-term (72 h) treatment with dcOC at different concentrations (0.3, 3, and 10 ng/mL groups, $P < 0.01$; 30 ng/mL group, $P < 0.05$). DcOC triggered Akt phosphorylation in a dose-dependent manner, and the most effective stimulatory concentration of dcOC for short-term (1 h) was 3 ng/mL ($P < 0.01$). LY294002 abolished the dcOC-mediated (1 h) promotion of Akt phosphorylation and glucose uptake without affecting GLUT1 protein expression. Long-term dcOC stimulation triggered Akt phosphorylation and increased the protein levels of HIF-1α, GLUT1, and Runx2 in a dose-dependent manner. Inhibition of HIF-1α with siRNA abolished the dcOC-mediated glucose uptake and substantially decreased GLUT1 protein expression. DcOC interven-tion promoted cell proliferation in a time- and dose-dependent manner as determined by the CCK-8 assay. Treatment with both 3 ng/mL and 10 ng/mL dcOC affected the ALP activity in MG63 cells after 72 h ($P < 0.01$).

CONCLUSION

Short- and long-term dcOC treatment can increase glucose uptake and affect proliferation and ALP activity in MG63 cells. This effect may occur through the PI3K/Akt, HIF-1α, and GLUT1 signaling factors.

Key Words: Decarboxylated osteocalcin; Osteoblast; Glucose uptake; Glucose transporter 1; Type 2 diabetes

Core Tip: Uncarboxylated osteocalcin (GluOC) has been reported to improve glucose metabolism and prevent type 2 diabetes. GluOC can increase the glucose uptake in a variety of cells. In this study, MG63 cells were treated with different concentrations of decarboxylated osteocalcin (dcOC) for 1 h and 72 h to observe the changes in glucose uptake, proliferation, and alkaline phosphatase (ALP) activity, as well as possible signaling pathway. Short- or long-term intervention with dcOC *in vitro* can increase glucose uptake and promote the proliferation and ALP activity of MG63 cells. This effect may occur through the PI3K/Akt, hypoxia-inducible factor 1 alpha, and glucose transporter 1 signaling factors.

Citation: Jin S, Chang XC, Wen J, Yang J, Ao N, Zhang KY, Suo LN, Du J. Decarboxylated osteocalcin, a possible drug for type 2 diabetes, triggers glucose uptake in MG63 cells. *World J Diabetes* 2021; 12(7): 1102-1115
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1102.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1102

INTRODUCTION

Osteocalcin (OC), synthesized by mature osteoblasts, is one of the most abundant noncollagen proteins in bone[1]. In the circulation, the concentration of OC is a measure of bone formation[2]. In osteoblasts, after protein translation in the endoplasmic reticulum, OC is posttranslationally modified by the addition of carboxyl groups at glutamyl (Glu) residues at positions 17, 21, and 24 *via* γ-Glu carboxylase, thereby facilitating its binding to hydroxyapatite in the bone matrix[1,3]. However, OC containing one or more uncarboxylated Glu residues (most commonly Glu17) is referred to as undercarboxylated OC (ucOC). Carboxylated OC can also be decar-boxylated at Glu residues under acidic conditions and is thus converted to decarboxylated OC (dcOC). Together, ucOC and dcOC are referred to as uncarboxylated OC (GluOC) and are closely related to energy metabolism[4]. To date, studies have confirmed that G protein-coupled receptor family C group 6 subtype A (GPRC6A), the receptor for carboxylated OC and GluOC, participates in the physio-

human beings or animals, the study was not reviewed and approved by any institutional review board.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: China

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 21, 2021
Peer-review started: January 21, 2021
First decision: March 16, 2021
Revised: April 5, 2021
Accepted: June 4, 2021
Article in press: June 4, 2021
Published online: July 15, 2021

P-Reviewer: Evans RW
S-Editor: Gao CC
L-Editor: Wang TQ
P-Editor: Ma YJ

logical response and glucose metabolism induced by GluOC[5-7].

Previous studies indicated that GluOC can improve glucose metabolism, prevent type 2 diabetes, and decrease the severity of obesity in mice with type 2 diabetes[8,9]. GluOC has great potential to become a new drug for the treatment of type 2 diabetes in the future. GluOC was also shown to promote glucose uptake in adipocytes, C2C12 myotubes, and muscles[10-13]. However, both the direct effects of GluOC on glucose uptake in bone tissues and the underlying mechanisms remain unexplored.

A previous study showed that the energy required for osteoblast proliferation and differentiation is produced mainly *via* glucose metabolism[14], and glucose transporter 1 (GLUT1) is the major glucose transporter[15]. GLUT1-mediated glucose uptake in osteoblasts occurs independently of insulin[14]. Moreover, GLUT1 may participate in osteosarcoma biology[16,17]. Activation of the PI3K/Akt and hypoxia-inducible factor 1 alpha (HIF-1α) signaling pathways is closely related to glucose uptake mediated by GLUT1[18,19]. Idelevich *et al*[20] found that the glucose uptake rate of mouse chondrocyte precursor cells (ADTC5 cells) overexpressing OC was increased. The siRNA-mediated silencing of HIF-1α abolished the effect of OC in upregulating the protein and mRNA expression of GLUT1, suggesting that HIF-1α mediates the effect of OC on upregulating the expression of GLUT1 in ADTC5 cells[20].

Herein, we treated MG63 cells, a human osteoblast-like osteosarcoma cell line, with dcOC for 1 h and 72 h *in vitro* in the absence of insulin to confirm whether dcOC affects glucose uptake and to verify whether PI3K/Akt and HIF-1α are involved in this process.

MATERIALS AND METHODS

Materials

MG63, HEK-293, and Jurkat cells were purchased from American Type Culture Collection. Synthetic human dcOC was purchased from Creative BioMart (United States). Antibodies specific for p-AKT (Ser473), AKT, HIF-1α, GLUT1, and Runx2 were purchased from Cell Signaling Technology.

Cell culture and treatment

Synthetic human dcOC was freshly resuspended and diluted in phosphate-buffered saline to the desired final concentrations before the experiments. MG63, HEK-293, and Jurkat cells were cultured in low-glucose Dulbecco's modified Eagle's medium (L-DMEM; 1000 mg/L glucose, HyClone, United States), high-glucose Dulbecco's modified Eagle's medium (H-DMEM; 4500 mg/L glucose, HyClone), and complete RPMI 1640 medium (HyClone), respectively. All media were supplemented with 10% fetal bovine serum (FBS, HyClone, United States) and 1% Pen-Strep, and cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2. When the cells were 70% confluent, the medium was replaced with L-DMEM containing 4% FBS and different concentrations of dcOC (0, 0.3, 3, 10, and 30 ng/mL). When MG63 cells were treated with different concentrations of dcOC solution for 72 h, the culture medium was changed daily. After cells were treated for 1 h or 72 h, glucose uptake and Western blot analyses were performed to determine the optimal concentration of dcOC.

For the PI3K inhibition assay, cells were treated with the PI3K inhibitor LY294002 (10 μmol/L, Cayman Chemical Inc., Michigan, United States) or 0.1% dimethyl sulfoxide as the vehicle control for 20 min and then treated with dcOC for 1 h.

Glucose uptake assay with 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose

A glucose uptake assay was performed according to a previously described method with minor modifications[21]. MG63 cells were plated at a density of 1×10^6 cells per well (for the 1 h dcOC treatment) or 1×10^5 cells per well (for the 72 h dcOC treatment) in 6-well plates and used at subconfluence. After 24 h of incubation, the culture medium was replaced with 2.5 mL of culture medium containing various concentrations of dcOC (0, 0.3, 3, 10, or 30 ng/mL) and incubated for 1 h or 72 h. The medium was then removed, and the cells were washed twice with precooled Krebs Ringer buffer (KRB). Fresh culture medium containing 100 μmol/L 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG, Creative BioMart Inc., New York, NY, United States), a fluorescent glucose analog, was added to the cells and incubated at 37 °C for 30 min in a humidified atmosphere with 5% CO_2. 2-NBDG uptake was stopped

by removal of the incubation medium, and the cells were washed twice with precooled KRB. The cells were trypsinized and resuspended in 2.0 mL of precooled fresh growth medium. For each measurement, data from 10000 single-cell events were recorded by flow cytometry (FACSCalibur, BD, United States). Second, cells were plated at a density of 1 × 10^5 cells per well in 6-well plates and used at subconfluence after 24 h of incubation. The culture medium was then removed from each well and replaced with 2.5 mL of culture medium containing dcOC (0, 0.3, 3, 10, or 30 ng/mL) for 72 h of preincubation. The subsequent steps were the same as those listed above. The optimal concentration at dcOC affected glucose uptake was determined by these experiments. The experiment was performed in triplicate, and the experimental data were analyzed using FlowJo software. The geometric mean was used to determine the mean fluorescence intensity (MFI). The average fluorescence intensity of each group was compared with that of the control group to obtain the normalized fluorescence intensity (AU), which indicated the glucose uptake ability of MG63 cells.

SiRNA-mediated silencing of HIF-1α

MG63 cells were grown and transfected with either HIF-1α siRNA (Sangon Biotech, Shanghai, China) or negative control siRNA using the transfection reagent Lipofectamine 2000 (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's instructions. After transfection, the medium was replaced with regular medium, and the cells were treated with dcOC for 72 h. Glucose uptake and Western blot assays were then performed.

PI3K inhibition assay

MG63 cells were treated with the PI3K inhibitor LY294002 (10 μmol/L) for 30 min and then with dcOC for 1 h. Then, glucose uptake and Western blot assays were performed in triplicate. The glucose uptake data were analyzed using FlowJo software. The geometric mean was used to determine the MFI.

Western blot analysis

The levels of GPRC6A, p-AKT (Ser473), AKT, HIF-1α, GLUT1, and Runx2 in MG63 cells were determined. For analysis of GPRC6A expression, HEK-293 cells were used as the negative control[22], and Jurkat cells were used as the positive control. Cell samples were homogenized on ice in lysis buffer containing protease and phosphatase inhibitors. A bicinchoninic acid protein assay was used to determine the protein concentration according to the manufacturer's instructions (Beyotime Institute of Biotechnology, China). All steps were performed as previously described[23]. Immunoblotting was performed using primary antibodies against GPRC6A (Absin Bioscience Inc., China) and horseradish peroxidase-conjugated anti-rabbit and anti-mouse secondary antibodies (Cell Signaling Technology). Immunoreactions were visualized with enhanced chemiluminescence reagents (GE Healthcare, UK, Ltd.). The band intensities were determined using ImageJ analysis software (NIH).

Cell proliferation assay

A Cell Counting Kit-8 (CCK-8, Tongren, China) assay was used to assess the effects of dcOC on MG63 cell proliferation. In brief, cells were plated in 96-well plates (4000 cells per well) in L-DMEM supplemented with 4% serum containing normal (5.5 mmol/L) levels of glucose and treated with various concentrations of dcOC (0, 0.3, 3, 10, and 30 ng/mL) for 24, 48, or 72 h. The absorbance values were read at 450 nm using an automated microplate reader (Power Waves XS, BioTek, United States).

Alkaline phosphatase activity assay

The rate of p-nitrophenyl phosphate (Sigma) hydrolysis was analyzed to measure alkaline phosphatase (ALP) activity. Cells were plated in 24-well plates at a density of 1 × 10^5 cells per well and incubated overnight. Then, the cells were incubated with solutions containing various concentrations of dcOC for 72 h. The medium was removed, and 500 μL of 0.1% Triton X-100 (Sigma) was added to each well. Cells were frozen at -70 °C, thawed at 37 °C, and centrifuged at 14000 *g* and 4 °C for 10 min. The supernatant was collected for ALP activity measurement. The absorbance values at 405 nm were read on a microplate reader (Power Waves XS, BioTek, United States). The Bradford method was used to determine the protein concentration.

Statistical analysis

Every experiment was performed at least three times. Data are presented as the mean ± SD. The results were analyzed by one-way analysis of variance (ANOVA) followed

by the LSD method for multiple comparisons using the statistical software SPSS 22.0. A P value of < 0.05 was considered statistically significant.

RESULTS

Evidence of GPRC6A receptor expression in MG63 cells

Previous studies have indicated that GPRC6A is expressed in bones and osteoblasts [24] of mice. We examined the expression of GPRC6A in MG63 cells by Western blot. Because previous experiments confirmed that HEK-293 cells lack GPRC6A expression [5], they were selected as the negative control, while Jurkat cells served as the positive control. Western blot analysis confirmed that MG63 cells expressed the GPRC6A receptor (Figure 1), thereby indicating that the roles of dcOC in MG63 cells could be examined.

DcOC increases glucose uptake in MG63 cells

The normalized fluorescence intensity (AU) of MG63 cells was significantly increased compared with that of the paired control cells after short-term (1 h) treatment with dcOC at different concentrations (0.3, 3, and 10 ng/mL groups, P < 0.01; 30 ng/mL group, P < 0.05; Figure 2A and B). In addition, 3 ng/mL was determined to be the optimal concentration for short-term (1 h) stimulation. Treatment with 3 ng/mL dcOC (1 h) increased glucose intake by 79% compared with that in the control group (P < 0.01; Figure 2B). In addition, the normalized fluorescence intensity (AU) of MG63 cells was significantly increased compared with that of paired control cells after long-term (72 h) treatment with dcOC at different concentrations (0.3, 3, and 10 ng/mL groups, P < 0.01; 30 ng/mL group, P < 0.05; Figure 2C and D). Additionally, 10 ng/mL was determined to be the optimal concentration for long-term (72 h) stimulation. Treatment with 10 ng/mL dcOC (72 h) increased glucose intake by 81% compared with that in the control group (P < 0.01; Figure 2D).

Involvement of the PI3K/Akt pathway in the promotional effect of dcOC on glucose uptake after short-term stimulation

As shown in Figure 3A and B, dcOC triggered Akt phosphorylation in a dose-dependent manner, and the most effective stimulatory concentration was 3 ng/mL in the short term (1 h) (P < 0.01). The protein expression of p-Akt (Ser473) in the 3 ng/mL group was increased by 2.99-fold compared to that in the 0 ng/mL group, as assessed relative to the level of Akt, after 1 h of dcOC stimulation (P < 0.01; Figure 3B). However, no significant changes were observed in the expression levels of GPRC6A, HIF-1α, GLUT1, or Runx2 as assessed relative to the level of β-actin (Figure 3B). Moreover, inhibition of PI3K with LY294002 abolished the dcOC-mediated promotion of Akt phosphorylation (Figure 3C and D) and 2-NBDG uptake (Figure 3E and F) without affecting GLUT1 protein expression (Figure 3C and D), indicating that PI3K/Akt signaling is involved in the enhancement of dcOC-induced glucose uptake for a short period (1 h).

Involvement of HIF-1α in the promotional effect of dcOC on glucose uptake after long-term stimulation

Long-term dcOC stimulation not only triggered Akt phosphorylation but also increased the protein levels of HIF-1α, GLUT1, and Runx2 in a dose-dependent manner (Figure 4B), whereas no increase in the expression level of GPRC6A was observed (Figure 4A and B). The most effective stimulatory concentration of dcOC was 10 ng/mL. The Western blot results indicated that the protein expression of p-Akt in the 10 ng/mL group was increased by 1.93-fold compared to that in the 0 ng/mL group, as assessed relative to the level of Akt, after 72 h of dcOC stimulation (P < 0.01, Figure 4B). The protein expression of HIF-1α, GLUT1, and Runx2 in the 10 ng/mL group was increased by 2.32-fold (P < 0.01, Figure 4B), 1.40-fold (P < 0.05, Figure 4B), and 2.06-fold (P < 0.01, Figure 4B), respectively, compared to that in the 0 ng/mL group, as assessed relative to the level of β-actin. Then, HIF-1α siRNA was used to decrease the HIF-1α protein levels in MG63 cells (P < 0.05, *vs* the control group, Figure 4D). Inhibition of HIF-1α with siRNA abolished dcOC-mediated 2-NBDG uptake (Figure 4E and F) and substantially decreased the GLUT1 protein expression (Figure 4C and D), indicating that HIF-1α signaling is involved in the promotional effect of dcOC on glucose uptake *via* GLUT1 modulation.

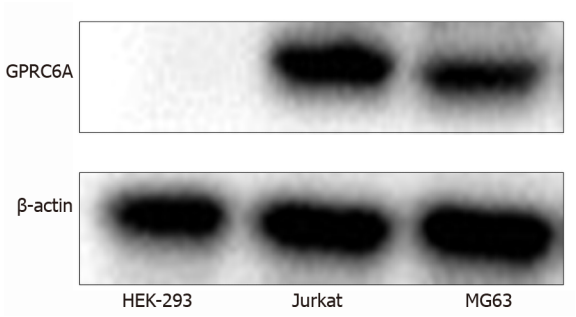

Figure 1 Western blot analysis demonstrating the protein expression of G protein-coupled receptor family C group 6 subtype A in MG63 cells. HEK-293 cells were selected as the negative control cells, and Jurkat cells were used as the positive control cells. GPRC6A: G protein-coupled receptor family C group 6 subtype A.

Biological effect of dcOC on MG63 cells

According to the cell proliferation assay results, the average cell counts for each day were plotted as a histogram. In the presence of dcOC, the number of cells was increased at 24, 48, and 72 h (Figure 5A). These results demonstrate that dcOC enhances MG63 cell proliferation in a time- and dose-dependent manner. DcOC had the most marked effect on proliferation at a concentration of 10 ng/mL (P <0.01, Figure 5A). The ALP activity assay results showed that treatment with either 3 ng/mL or 10 ng/mL dcOC affected ALP activity in MG63 cells after 72 h (P < 0.01, Figure 5B).

DISCUSSION

An increasing number of studies have shown that GluOC is closely related to energy metabolism[25,26]. *In vitro* experiments showed that different doses of GluOC could increase glucose uptake in various cells with or without insulin[11,12]. Glucose uptake is mediated by transmembrane GLUT proteins in cells[27]. Among these transporters, GLUT1 is closely related to the energy metabolism of tumor cells. The increase in glucose uptake by tumor cells is closely related to their proliferation[28]. Previous studies have shown that the expression of GLUT1 affects the prognosis of patients with osteosarcoma[29,30]. In addition, Fan *et al*[31] reported that interfering with GLUT1 expression may inhibit the proliferation of MG63 cells *in vitro*. Our study explored the relationship between dcOC, a type of GluOC, and glucose uptake in osteoblastic sarcoma cells *in vitro* and the possible association of this effect with GLUT1. Although MG63 cells synthesize GluOC, the level of GluOC synthesized by MG63 cells without retinoic acid intervention and lα,25-dihydroxycholecalciferol [lα,25(OH)2D], as shown in previous studies[32], was negligible compared with the level of exogenous dcOC in our study. In addition, the concentration of dcOC used in our study was confirmed to increase glucose uptake in the previous study[11].

As the receptor for GluOC, GPRC6A plays an important role in the regulation of glucose metabolism by GluOC in histiocytes[6,10]. The expression of GPRC6A in human osteoblasts was confirmed by scholars as early as 1998[33]. In the present study, we confirmed the expression of GPRC6A in MG63 cells (Figure 1), which provided a basis to study the effect of dcOC on the metabolic response. In our study, both short- and long-term dcOC intervention altered the glucose uptake by MG63 cells but did not change the protein level of GPRC6A. We speculate that dcOC may affect the signaling pathway downstream of GPRC6A by altering the protein conformation of GPRC6A, thereby affecting the glucose uptake of MG63 cells. However, further investigation is needed to confirm this hypothesis.

Hill *et al*[11] showed that short-term intervention with carboxylated osteocalcin in adipocytes could increase the glucose uptake and transport in a cell-based system (in an insulin-independent manner). GLUT1 is the main glucose transporter in osteoblasts [15]. Activation of the PI3K/Akt signaling pathway has been confirmed to be necessary for GLUT1 translocation, cell glucose uptake, and maintenance of cell surface GLUT1 Levels[18]. Application of a PI3K inhibitor can effectively inhibit the expression of GLUT1 on the cell membrane, thereby affecting the uptake of glucose in cells[34]. A previous study showed that ucOC could increase the phosphorylation of Akt in adipocytes in an insulin-independent manner[12]. In our study, short-term incubation (1 h) of MG63 cells with dcOC promoted glucose uptake under

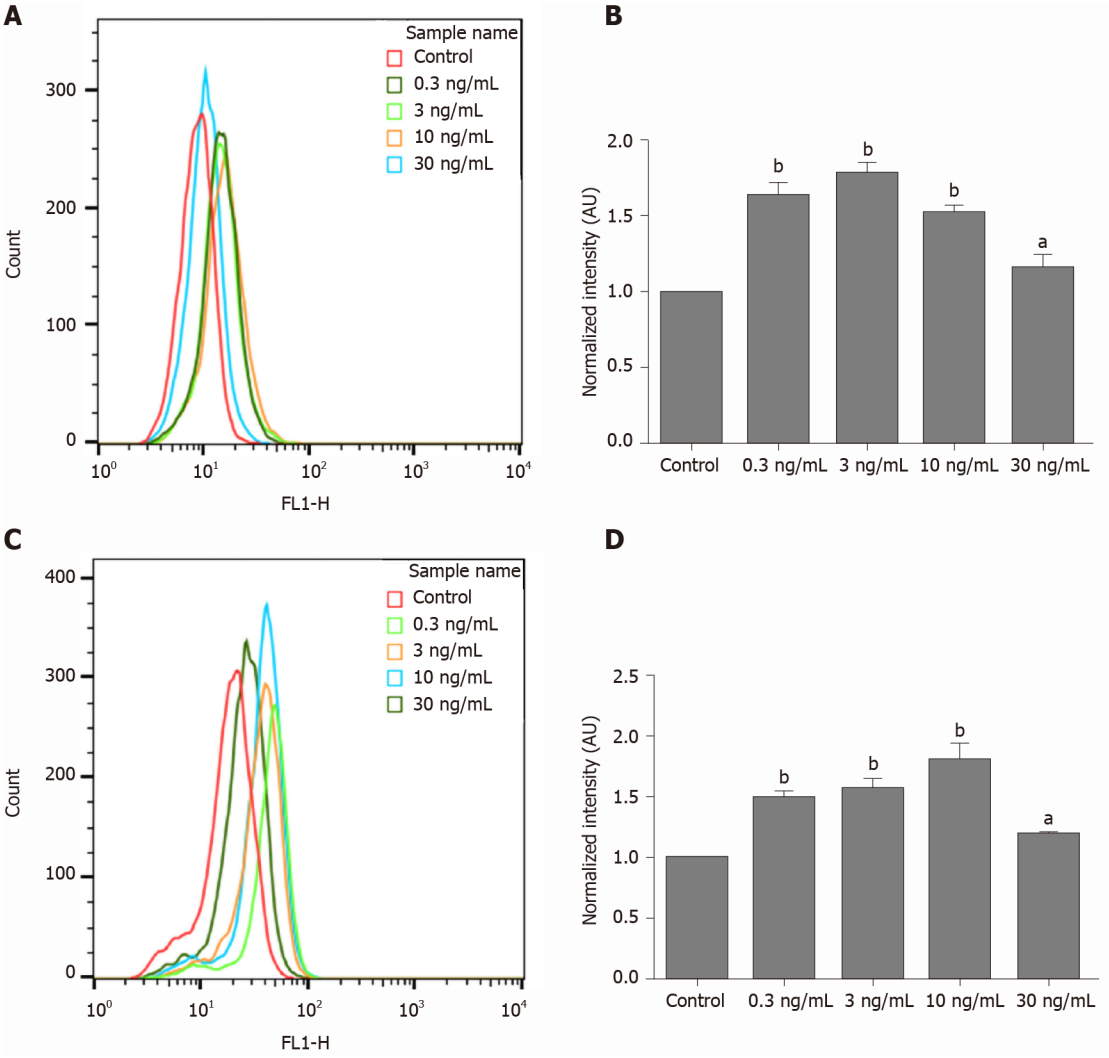

Figure 2 Flow cytometric analyses of 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose uptake by MG63 cells after decarboxylated osteocalcin stimulation. A: The distribution of fluorescence intensities which represented 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) uptake after decarboxylated osteocalcin (dcOC) stimulation for 1 h; B: The normalized fluorescence intensities corresponding to 2-NBDG uptake after dcOC stimulation for 1 h; C: Distribution of fluorescence intensities which represented 2-NBDG uptake after dcOC stimulation for 72 h; D: The normalized fluorescence intensity corresponding to 2-NBDG uptake after dcOC stimulation for 72 h. The red lines in the histograms in A and C indicate the control group (0 ng/mL), while the other lines indicate the groups treated with dcOC at different concentrations. The data are representative of three independent experiments performed in triplicate. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the control group.

physiological glucose conditions in an insulin-independent manner (Figure 2A and B). In addition, the phosphorylation of AKT was increased significantly in MG63 cells incubated with dcOC for a short time, although the GLUT1 expression was not affected (Figure 3A and B), suggesting that short-term dcOC treatment increases the glucose uptake rate, probably by regulating GLUT1 activity and transport *via* PI3K/Akt rather than by increasing the protein expression of GLUT1. Then, we used the PI3K inhibitor LY294002 to reduce the phosphorylation of Akt and found that the effect of dcOC on increasing glucose uptake was abolished (Figure 3C-F). This result confirmed that the PI3K/Akt signaling pathway participates in dcOC, promoting glucose uptake in MG63 cells. In addition to GLUT1, GLUT3 and GLUT4 have also been reported to be expressed in osteoblasts[15]. The increase in Akt phosphorylation is related to increases in GLUT3 and GLUT4 translocation and protein expression levels[35,36]. DcOC may increase glucose uptake by increasing the GLUT3 or GLUT4 translocation or protein expression in MG63 cells after short-term intervention, but further research is needed to confirm this hypothesis. Moreover, the expression of GPRC6A was not altered by short-term treatment with dcOC, suggesting that short-term dcOC intervention induces glucose uptake in MG63 cells by altering the activity or configuration of the receptor GPRC6A rather than its expression.

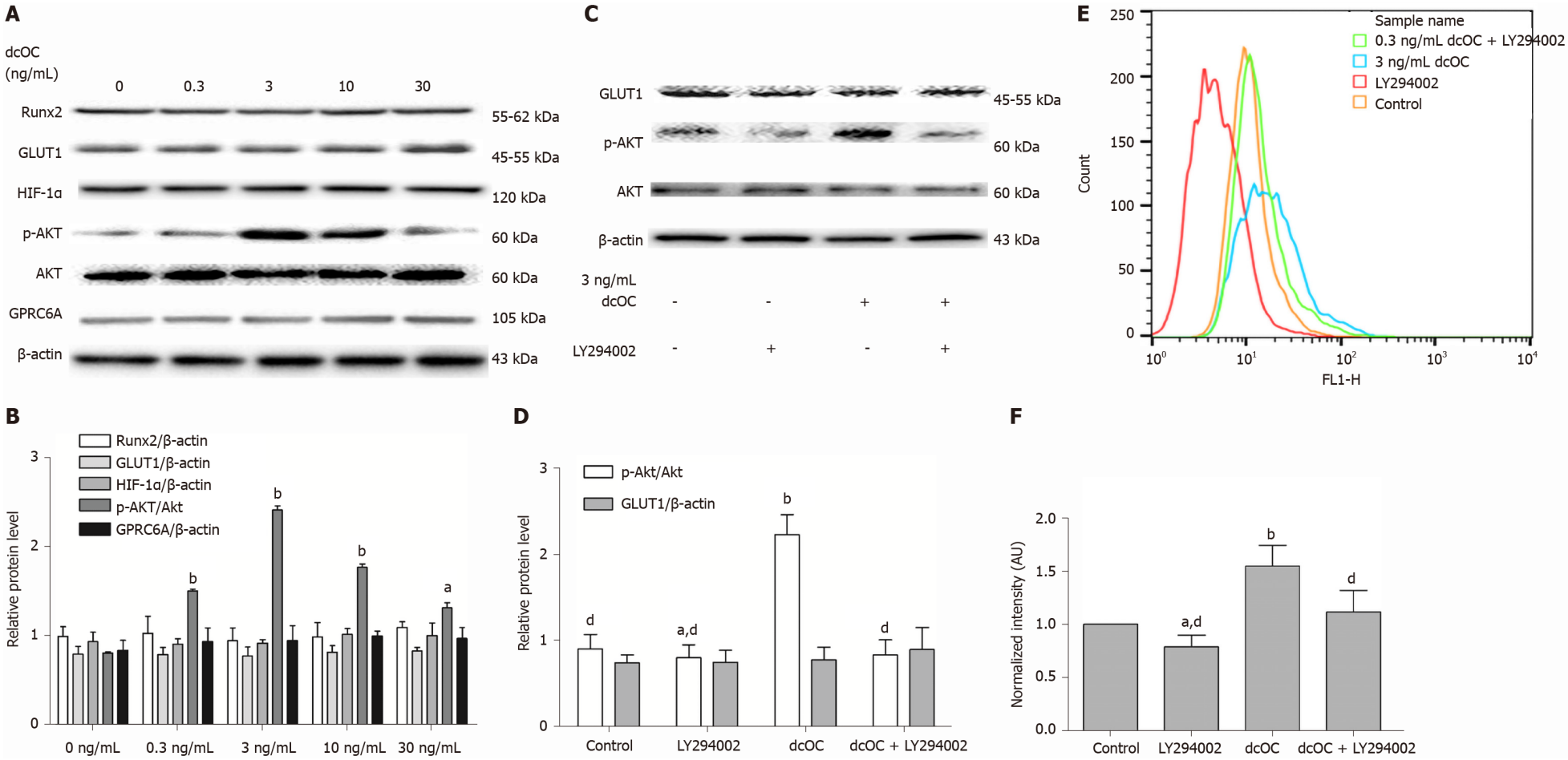

Figure 3 Promotional effect of decarboxylated osteocalcin on glucose uptake after short-term stimulation (1 h) in MG63 cells. A: Protein bands; B: Relative protein levels of GPRC6A (G protein-coupled receptor family C group 6 subtype A), p-Akt (Ser473)/Akt, hypoxia-inducible factor 1 alpha, glucose transporter 1 (GLUT1), and Runx2 after decarboxylated osteocalcin (dcOC) stimulation for 1 h at different concentrations. C: Protein bands; D: Relative protein levels of p-Akt (Ser473)/Akt and GLUT1. E: The distribution of fluorescence intensities which represented 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) uptake; F: The normalized fluorescence intensity corresponding to 2-NBDG uptake. The MG63 cells in C-F were treated with dcOC (3 ng/mL) for 1 h and (or) LY294002 (10 μmol/L) for 30 min. The data are representative of three independent experiments performed in triplicate. B: $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* untreated cells; D and F: $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the control group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* the dcOC-only group. dcOC: Decarboxylated osteocalcin; HIF-1α: Hypoxia-inducible factor 1 alpha; GLUT1: Glucose transporter 1; GPRC6A: G protein-coupled receptor family C group 6 subtype A.

Next, we validated the effect of glucose uptake on MG63 cells with long-term dcOC intervention (72 h). Similar to the short-term stimulation, the long-term stimulation of MG63 cells with dcOC also promoted glucose uptake under physiological glucose conditions in an insulin-independent manner (Figure 2C and D), accompanied by a

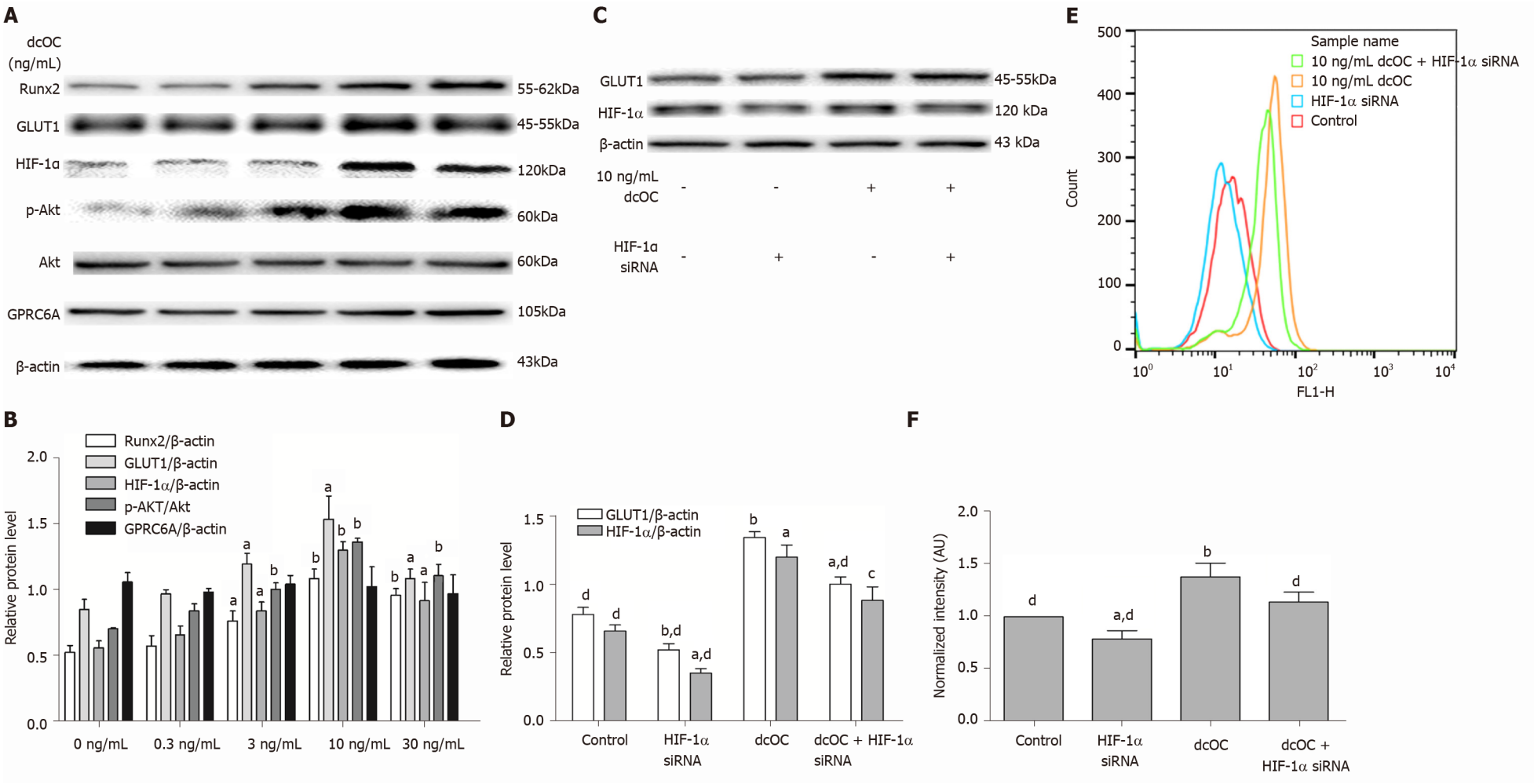

Figure 4 Promotional effect of decarboxylated osteocalcin on glucose uptake after long-term stimulation (72 h) in MG63 cells. A: Protein bands; B: Relative protein levels of GPRC6A (G protein-coupled receptor family C group 6 subtype A), p-Akt (Ser473)/Akt, hypoxia-inducible factor 1 alpha (HIF-1α), glucose transporter 1 (GLUT1), and Runx2 after decarboxylated osteocalcin (dcOC) stimulation for 72 h at different concentrations; C: Protein bands; D: Relative protein levels of HIF-1α and GLUT1; E: The distribution of fluorescence intensities which represented 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) uptake; F: The normalized fluorescence intensity corresponding to 2-NBDG uptake. The MG63 cells in C-F were treated with dcOC (10 ng/mL) 72 h and (or) were transfected with HIF-1α siRNA. The data are representative of three independent experiments performed in triplicate. B: $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* untreated cells; D and F: $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the control group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* the dcOC-only group. dcOC: Decarboxylated osteocalcin; HIF-1α: Hypoxia-inducible factor 1 alpha; GLUT1: Glucose transporter 1; GPRC6A: G protein-coupled receptor family C group 6 subtype A.

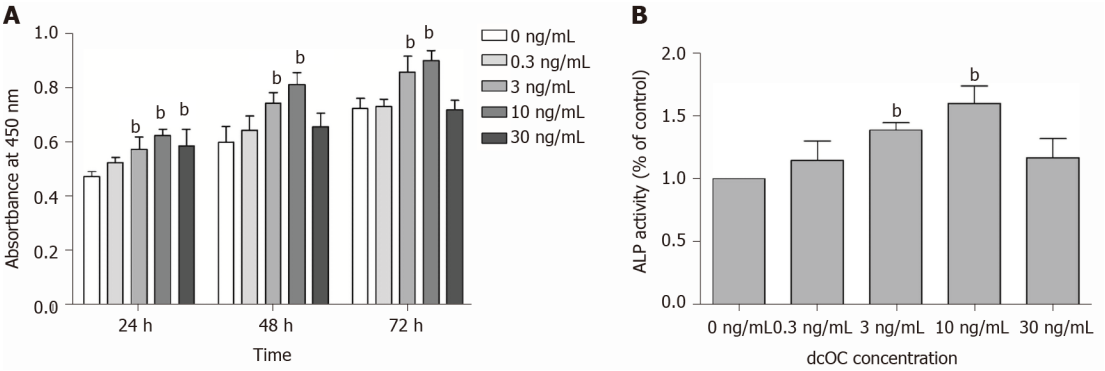

Figure 5 Effects of decarboxylated osteocalcin on the proliferation and alkaline phosphatase activity in MG63 cells. A: Histogram showing the effects of decarboxylated osteocalcin (dcOC) on MG63 cell proliferation. The absorbance value was measured at 24, 48, and 72 h; B: Alkaline phosphatase activity in MG63 cells after 72 h of incubation with different concentrations of dcOC. Significant differences ($P < 0.01$) were found between the dcOC treatment groups (3 ng/mL and 10 ng/mL) and the control group (0 ng/mL). The data are representative of three independent experiments performed in triplicate. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the control group (0 ng/mL). dcOC: Decarboxylated osteocalcin; ALP: Alkaline phosphatase.

significantly increased p-AKT level (Figure 4A and B). However, long-term stimulation significantly increased the protein expression levels of HIF-1α and GLUT1 in MG63 cells treated with different concentrations of dcOC, suggesting that dcOC can increase the glucose uptake rate, probably by regulating GLUT1 protein expression after incubation for an extended duration (Figure 4A and B). Then, we silenced HIF-1α *via* siRNA and found that the effect of dcOC on increasing glucose uptake was abolished, with decreased expression of HIF-1α and GLUT1 (Figure 4C-F). HIF-1 is a transcription factor that mediates the adaptive response, which enhances the energy metabolism of cancer cells. HIF-1 is composed of two subunits, HIF-1α and HIF-1β, among which HIF-1α determines the activity of HIF-1. HIF-1α is closely related to GLUT1 in tumor cells. For example, HIF-1α can upregulate the protein expression of GLUT1[37], and silencing or inhibiting HIF-1α in tumor cells can decrease the expression of GLUT1[38,39]. Previous studies have shown that HIF-1α plays an important role in the growth of osteosarcoma[40,41]. The results of our study suggested that dcOC promotes the glucose uptake in MG63 cells after incubation for an extended duration by upregulating the protein expression of GLUT1. Using HIF-1α siRNA, we demonstrated that GLUT1 expression induced by dcOC is dependent on HIF-1α in MG63 cells. Therefore, these results support a new perspective on the modulation of GLUT1 expression by HIF-1α in response to dcOC in MG63 cells.

Runx2 is an essential transcription factor for osteoblast differentiation, matrix production, and mineralization during bone formation[42]. It controls the expression of bone formation-related genes such as *OCN, ALP, BMP2*, and *BMP4*[43]. In this study, short-term intervention with dcOC in MG63 cells did not change the protein expression of Runx2 (Figure 3A and B), while long-term dcOC intervention significantly increased the protein expression of Runx2 (Figure 4A and B). The optimal concentration was consistent with that required to increase GLUT1 expression (10 ng/mL) (Figure 4A and B). Runx2 expression has been reported to be significantly increased in human osteosarcoma tissues and osteosarcoma cell lines[44]. Runx2 is closely related to osteosarcoma metastasis, and downregulation of Runx2 was found to inhibit osteosarcoma metastasis and invasion[45]. Here, we speculated that long-term intervention with dcOC influenced osteoblastic osteosarcoma cell metastasis and invasion by increasing glucose uptake, which was achieved by increasing the expression of GLUT1.

In our study, dcOC promoted the proliferation of MG63 cells (Figure 5A). Bone formation and osteoblast proliferation are energy-expensive processes, and the energy for osteoblast proliferation is generated mainly *via* glucose metabolism[14]. Because GLUT1 is the key glucose transporter regulating glucose uptake[46], we speculated that the ability of dcOC to increase MG63 cell proliferation is closely related to its ability to improve glucose metabolism.

ALP is a major enzyme expressed during the early maturation of osteoblasts. UcOC has been reported to increase ALP activity in bone marrow mesenchymal stem cells (BMSCs)[47]. In our study, the ALP activity in MG63 cells was increased after dcOC treatment for 72 h (Figure 5B), consistent with the results of a previous study. Moreover, previous studies have shown that increased serum ALP concentrations are indicative of a worse prognosis in osteosarcoma, correlating with shorter survival

times and disease-free intervals[48-50]. The results of our study indicated that dcOC might affect the prognosis of osteosarcoma patients by increasing the ALP activity in osteoblast-like osteosarcoma cells. However, the specific mechanism by which dcOC increases ALP activity is not clear and needs to be further investigated.

GluOC has been suggested to be closely related to energy metabolism and can increase glucose uptake in various cells[10-13]. In animal experiments, GluOC has been proven to reduce blood glucose levels and visceral fat in mice with type 2 diabetes[8,9]. GluOC is considered a potential agent for the treatment of type 2 diabetes and insulin resistance. The present study clearly showed that dcOC, a type of GluOC, promoted glucose uptake in MG63 cells *in vitro*. dcOC may affect the invasion and migration of MG63 cells and the prognosis of osteosarcoma patients by affecting the ALP activity and Runx2 expression in osteoblastic sarcoma cells. Thus, while considering dcOC as a potential treatment for type 2 diabetes, it is also necessary to be aware of its possible adverse effects on osteoblastic osteosarcoma.

CONCLUSION

DcOC can promote glucose uptake in MG63 cells *in vitro*. DcOC may affect the invasion and migration of MG63 cells and the prognosis of osteosarcoma patients by affecting ALP activity and Runx2 expression.

ARTICLE HIGHLIGHTS

Research background

Uncarboxylated osteocalcin (GluOC) has been reported to improve glucose metabolism, prevent type 2 diabetes, and decrease the severity of obesity in mice with type 2 diabetes. GluOC can increase glucose uptake in a variety of cells. GluOC has great potential to become a new drug for the treatment of type 2 diabetes in the future.

Research motivation

Glucose metabolism is the main source of energy for osteoblast proliferation and differentiation. However, both the direct effects of GluOC on glucose uptake in bone tissues and the underlying mechanisms remain unexplored.

Research objectives

To investigate the effects of decarboxylated osteocalcin (dcOC), a kind of GluOC, on glucose uptake in human osteoblast-like osteosarcoma cells and the possible signaling pathways involved.

Research methods

MG63 cells (human osteoblast-like osteosarcoma cells) were treated with dcOC (0, 0.3, 3, 10, or 30 ng/mL) for 1 and 72 h, and glucose uptake was measured by flow cytometry. The effect of dcOC on cell proliferation was measured with a CCK-8 assay, and alkaline phosphatase (ALP) enzyme activity was measured. PI3K was inhibited with LY294002, and hypoxia-inducible factor 1 alpha (HIF-1α) was silenced with siRNA. Then, the G protein-coupled receptor family C group 6 subtype A, total Akt, phosphorylated Akt, HIF-1α, and glucose transporter 1 (GLUT1) levels were measured by Western blot to elucidate the possible pathways by which dcOC modulates glucose uptake.

Research results

The glucose uptake of MG63 cells was significantly increased compared with that of the paired control cells after short-term (1 h) treatment and long-term (72 h) treatment with dcOC at different concentrations. LY294002 abolished the dcOC-mediated (1 h) promotion of Akt phosphorylation and glucose uptake without affecting GLUT1 protein expression. Long-term dcOC stimulation triggered Akt phosphorylation and increased the protein levels of HIF-1α, GLUT1, and Runx2 in a dose-dependent manner. Inhibition of HIF-1α abolished the dcOC-mediated glucose uptake and substantially decreased GLUT1 protein expression. DcOC intervention promoted cell proliferation in a time- and dose-dependent manner. Treatment with dcOC affected the ALP activity in MG63 cells.

Research conclusions

DcOC can promote glucose uptake in MG63 cells *in vitro*. DcOC may affect the invasion and migration of MG63 cells and the prognosis of osteosarcoma patients by affecting ALP activity and Runx2 expression.

Research perspectives

DcOC can promote glucose uptake in human osteoblast-like osteosarcoma cells. It is necessary to be aware of its possible adverse effects on osteoblastic osteosarcoma while considering dcOC as a potential treatment for type 2 diabetes.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Zhao YY and Bai BW for skillful technical assistance.

REFERENCES

1 **Neve A**, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. *J Cell Physiol* 2013; **228**: 1149-1153 [PMID: 23139068 DOI: 10.1002/jcp.24278]
2 **Booth SL**, Centi A, Smith SR, Gundberg C. The role of osteocalcin in human glucose metabolism: marker or mediator? *Nat Rev Endocrinol* 2013; **9**: 43-55 [PMID: 23147574 DOI: 10.1038/nrendo.2012.201]
3 **Hauschka PV**, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiol Rev* 1989; **69**: 990-1047 [PMID: 2664828 DOI: 10.1152/physrev.1989.69.3.990]
4 **Ferron M**, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, Ducy P, Karsenty G. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell* 2010; **142**: 296-308 [PMID: 20655470 DOI: 10.1016/j.cell.2010.06.003]
5 **Pi M**, Wu Y, Quarles LD. GPRC6A mediates responses to osteocalcin in β-cells *in vitro* and pancreas in vivo. *J Bone Miner Res* 2011; **26**: 1680-1683 [PMID: 21425331 DOI: 10.1002/jbmr.390]
6 **Wei J**, Hanna T, Suda N, Karsenty G, Ducy P. Osteocalcin promotes β-cell proliferation during development and adulthood through Gprc6a. *Diabetes* 2014; **63**: 1021-1031 [PMID: 24009262 DOI: 10.2337/db13-0887]
7 **Liu J**, Yang J. Uncarboxylated osteocalcin inhibits high glucose-induced ROS production and stimulates osteoblastic differentiation by preventing the activation of PI3K/Akt in MC3T3-E1 cells. *Int J Mol Med* 2016; **37**: 173-181 [PMID: 26719856 DOI: 10.3892/ijmm.2015.2412]
8 **Ferron M**, McKee MD, Levine RL, Ducy P, Karsenty G. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. *Bone* 2012; **50**: 568-575 [PMID: 21550430 DOI: 10.1016/j.bone.2011.04.017]
9 **Ferron M**, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *Proc Natl Acad Sci USA* 2008; **105**: 5266-5270 [PMID: 18362359 DOI: 10.1073/pnas.0711119105]
10 **Tsuka S**, Aonuma F, Higashi S, Ohsumi T, Nagano K, Mizokami A, Kawakubo-Yasukochi T, Masaki C, Hosokawa R, Hirata M, Takeuchi H. Promotion of insulin-induced glucose uptake in C2C12 myotubes by osteocalcin. *Biochem Biophys Res Commun* 2015; **459**: 437-442 [PMID: 25735975 DOI: 10.1016/j.bbrc.2015.02.123]
11 **Hill HS**, Grams J, Walton RG, Liu J, Moellering DR, Garvey WT. Carboxylated and uncarboxylated forms of osteocalcin directly modulate the glucose transport system and inflammation in adipocytes. *Horm Metab Res* 2014; **46**: 341-347 [PMID: 24554534 DOI: 10.1055/s-0034-1368709]
12 **Guedes JAC**, Esteves JV, Morais MR, Zorn TM, Furuya DT. Osteocalcin improves insulin resistance and inflammation in obese mice: Participation of white adipose tissue and bone. *Bone* 2018; **115**: 68-82 [PMID: 29183784 DOI: 10.1016/j.bone.2017.11.020]
13 **Lin X**, Parker L, Mclennan E, Zhang X, Hayes A, McConell G, Brennan-Speranza TC, Levinger I. Uncarboxylated Osteocalcin Enhances Glucose Uptake Ex Vivo in Insulin-Stimulated Mouse Oxidative But Not Glycolytic Muscle. *Calcif Tissue Int* 2018; **103**: 198-205 [PMID: 29427234 DOI: 10.1007/s00223-018-0400-x]
14 **Wei J**, Shimazu J, Makinistoglu MP, Maurizi A, Kajimura D, Zong H, Takarada T, Lezaki T, Pessin JE, Hinoi E, Karsenty G. Glucose Uptake and Runx2 Synergize to Orchestrate Osteoblast Differentiation and Bone Formation. *Cell* 2015; **161**: 1576-1591 [PMID: 26091038 DOI: 10.1016/j.cell.2015.05.029]
15 **Zoidis E**, Ghirlanda-Keller C, Schmid C. Stimulation of glucose transport in osteoblastic cells by parathyroid hormone and insulin-like growth factor I. *Mol Cell Biochem* 2011; **348**: 33-42 [PMID: 21076856 DOI: 10.1007/s11010-010-0634-z]
16 **Chen R**, Lin J, Yan W, Chen D. miR-522-3p Promotes Osteosarcoma Cell Growth By Regulating

Glucose Uptake And GLUT1 Expression. *Onco Targets Ther* 2019; **12**: 9053-9058 [PMID: 31802912 DOI: 10.2147/OTT.S217324]

17 **Lin S**, Zhu B, Huang G, Zeng Q, Wang C. Microvesicles derived from human bone marrow mesenchymal stem cells promote U2OS cell growth under hypoxia: the role of PI3K/AKT and HIF-1α. *Hum Cell* 2019; **32**: 64-74 [PMID: 30506278 DOI: 10.1007/s13577-018-0224-z]

18 **Wieman HL**, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake *via* phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. *Mol Biol Cell* 2007; **18**: 1437-1446 [PMID: 17301289 DOI: 10.1091/mbc.e06-07-0593]

19 **Heydarzadeh S**, Moshtaghie AA, Daneshpoor M, Hedayati M. Regulators of glucose uptake in thyroid cancer cell lines. *Cell Commun Signal* 2020; **18**: 83 [PMID: 32493394 DOI: 10.1186/s12964-020-00586-x]

20 **Idelevich A**, Rais Y, Monsonego-Ornan E. Bone Gla protein increases HIF-1alpha-dependent glucose metabolism and induces cartilage and vascular calcification. *Arterioscler Thromb Vasc Biol* 2011; **31**: e55-e71 [PMID: 21757657 DOI: 10.1161/ATVBAHA.111.230904]

21 **Wang MS**, Luo Z, Nitin N. Rapid assessment of drug response in cancer cells using microwell array and molecular imaging. *Anal Bioanal Chem* 2014; **406**: 4195-4206 [PMID: 24760393 DOI: 10.1007/s00216-014-7759-y]

22 **Pi M**, Parrill AL, Quarles LD. GPRC6A mediates the non-genomic effects of steroids. *J Biol Chem* 2010; **285**: 39953-39964 [PMID: 20947496 DOI: 10.1074/jbc.M110.158063]

23 **Guo Q**, Li H, Xu L, Wu S, Sun H, Zhou B. Undercarboxylated osteocalcin reverts insulin resistance induced by endoplasmic reticulum stress in human umbilical vein endothelial cells. *Sci Rep* 2017; **7**: 46 [PMID: 28246389 DOI: 10.1038/s41598-017-00163-2]

24 **Pi M**, Faber P, Ekema G, Jackson PD, Ting A, Wang N, Fontilla-Poole M, Mays RW, Brunden KR, Harrington JJ, Quarles LD. Identification of a novel extracellular cation-sensing G-protein-coupled receptor. *J Biol Chem* 2005; **280**: 40201-40209 [PMID: 16199532 DOI: 10.1074/jbc.M505186200]

25 **Lacombe J**, Karsenty G, Ferron M. In vivo analysis of the contribution of bone resorption to the control of glucose metabolism in mice. *Mol Metab* 2013; **2**: 498-504 [PMID: 24327965 DOI: 10.1016/j.molmet.2013.08.004]

26 **Yasutake Y**, Mizokami A, Kawakubo-Yasukochi T, Chishaki S, Takahashi I, Takeuchi H, Hirata M. Long-term oral administration of osteocalcin induces insulin resistance in male mice fed a high-fat, high-sucrose diet. *Am J Physiol Endocrinol Metab* 2016; **310**: E662-E675 [PMID: 26884384 DOI: 10.1152/ajpendo.00334.2015]

27 **Flier JS**, Mueckler MM, Usher P, Lodish HF. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science* 1987; **235**: 1492-1495 [PMID: 3103217 DOI: 10.1126/science.3103217]

28 **Hatanaka M**. Transport of sugars in tumor cell membranes. *Biochim Biophys Acta* 1974; **355**: 77-104 [PMID: 4371599 DOI: 10.1016/0304-419x(74)90008-0]

29 **Endo M**, Tateishi U, Seki K, Yamaguchi U, Nakatani F, Kawai A, Chuman H, Beppu Y. Prognostic implications of glucose transporter protein-1 (glut-1) overexpression in bone and soft-tissue sarcomas. *Jpn J Clin Oncol* 2007; **37**: 955-960 [PMID: 18211986 DOI: 10.1093/jjco/hym125]

30 **Schwartzenberg-Bar-Yoseph F**, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Cancer Res* 2004; **64**: 2627-2633 [PMID: 15059920 DOI: 10.1158/0008-5472.can-03-0846]

31 **Fan J**, Zhou JQ, Yu GR, Lu DD. Glucose transporter protein 1-targeted RNA interference inhibits growth and invasion of the osteosarcoma cell line MG63 in vitro. *Cancer Biother Radiopharm* 2010; **25**: 521-527 [PMID: 20854211 DOI: 10.1089/cbr.2010.0784]

32 **Szulc P**, Delmas PD. Influence of vitamin D and retinoids on the gammacarboxylation of osteocalcin in human osteosarcoma MG63 cells. *Bone* 1996; **19**: 615-620 [PMID: 8968028 DOI: 10.1016/s8756-3282(96)00265-7]

33 **Divieti P**, Lanske B, Kronenberg HM, Bringhurst FR. Conditionally immortalized murine osteoblasts lacking the type 1 PTH/PTHrP receptor. *J Bone Miner Res* 1998; **13**: 1835-1845 [PMID: 9844101 DOI: 10.1359/jbmr.1998.13.12.1835]

34 **Makinoshima H**, Takita M, Saruwatari K, Umemura S, Obata Y, Ishii G, Matsumoto S, Sugiyama E, Ochiai A, Abe R, Goto K, Esumi H, Tsuchihara K. Signaling through the Phosphatidylinositol 3-Kinase (PI3K)/Mammalian Target of Rapamycin (mTOR) Axis Is Responsible for Aerobic Glycolysis mediated by Glucose Transporter in Epidermal Growth Factor Receptor (EGFR)-mutated Lung Adenocarcinoma. *J Biol Chem* 2015; **290**: 17495-17504 [PMID: 26023239 DOI: 10.1074/jbc.M115.660498]

35 **Zaulkffali AS**, Md Razip NN, Syed Alwi SS, Abd Jalil A, Abd Mutalib MS, Gopalsamy B, Chang SK, Zainal Z, Ibrahim NN, Zakaria ZA, Khaza'ai H. Vitamins D and E Stimulate the *PI3K-AKT* Signalling Pathway in Insulin-Resistant SK-N-SH Neuronal Cells. *Nutrients* 2019; **11** [PMID: 31635074 DOI: 10.3390/nu11102525]

36 **Griffith CM**, Macklin LN, Cai Y, Sharp AA, Yan XX, Reagan LP, Strader AD, Rose GM, Patrylo PR. Impaired Glucose Tolerance and Reduced Plasma Insulin Precede Decreased AKT Phosphorylation and GLUT3 Translocation in the Hippocampus of Old 3xTg-AD Mice. *J Alzheimers Dis* 2019; **68**: 809-837 [PMID: 30775979 DOI: 10.3233/JAD-180707]

37 **Song K**, Li M, Xu XJ, Xuan L, Huang GN, Song XL, Liu QF. HIF-1α and GLUT1 gene expression is associated with chemoresistance of acute myeloid leukemia. *Asian Pac J Cancer Prev* 2014; **15**: 1823-1829 [PMID: 24641416 DOI: 10.7314/apjcp.2014.15.4.1823]

38 **Askenazi DJ**, Koralkar R, Levitan EB, Goldstein SL, Devarajan P, Khandrika S, Mehta RL, Ambalavanan N. Baseline values of candidate urine acute kidney injury biomarkers vary by gestational age in premature infants. *Pediatr Res* 2011; **70**: 302-306 [PMID: 21646940 DOI: 10.1203/PDR.0b013e3182275164]

39 **Ferrer CM**, Lynch TP, Sodi VL, Falcone JN, Schwab LP, Peacock DL, Vocadlo DJ, Seagroves TN, Reginato MJ. O-GlcNAcylation regulates cancer metabolism and survival stress signaling *via* regulation of the HIF-1 pathway. *Mol Cell* 2014; **54**: 820-831 [PMID: 24857547 DOI: 10.1016/j.molcel.2014.04.026]

40 **Guo M**, Cai C, Zhao G, Qiu X, Zhao H, Ma Q, Tian L, Li X, Hu Y, Liao B, Ma B, Fan Q. Hypoxia promotes migration and induces CXCR4 expression *via* HIF-1α activation in human osteosarcoma. *PLoS One* 2014; **9**: e90518 [PMID: 24618817 DOI: 10.1371/journal.pone.0090518]

41 **Zhang Y**, Cheng H, Li W, Wu H, Yang Y. Highly-expressed P2X7 receptor promotes growth and metastasis of human HOS/MNNG osteosarcoma cells *via* PI3K/Akt/GSK3β/β-catenin and mTOR/HIF1α/VEGF signaling. *Int J Cancer* 2019; **145**: 1068-1082 [PMID: 30761524 DOI: 10.1002/ijc.32207]

42 **Liu TM**, Lee EH. Transcriptional regulatory cascades in Runx2-dependent bone development. *Tissue Eng Part B Rev* 2013; **19**: 254-263 [PMID: 23150948 DOI: 10.1089/ten.TEB.2012.0527]

43 **Molagoda IMN**, Karunarathne WAHM, Choi YH, Park EK, Jeon YJ, Lee BJ, Kang CH, Kim GY. Fermented Oyster Extract Promotes Osteoblast Differentiation by Activating the Wnt/β-Catenin Signaling Pathway, Leading to Bone Formation. *Biomolecules* 2019; **9** [PMID: 31698882 DOI: 10.3390/biom9110711]

44 **Sadikovic B**, Thorner P, Chilton-Macneill S, Martin JW, Cervigne NK, Squire J, Zielenska M. Expression analysis of genes associated with human osteosarcoma tumors shows correlation of RUNX2 overexpression with poor response to chemotherapy. *BMC Cancer* 2010; **10**: 202 [PMID: 20465837 DOI: 10.1186/1471-2407-10-202]

45 **Xie Y**, Sun W, Deng Z, Zhu X, Hu C, Cai L. MiR-302b Suppresses Osteosarcoma Cell Migration and Invasion by Targeting Runx2. *Sci Rep* 2017; **7**: 13388 [PMID: 29042587 DOI: 10.1038/s41598-017-13353-9]

46 **Li W**, Deng Y, Feng B, Mak KK. Mst1/2 Kinases Modulate Glucose Uptake for Osteoblast Differentiation and Bone Formation. *J Bone Miner Res* 2018; **33**: 1183-1195 [PMID: 29474739 DOI: 10.1002/jbmr.3413]

47 **Liu Z**, Yang J. Uncarboxylated osteocalcin promotes osteogenic differentiation of mouse bone marrow-derived mesenchymal stem cells by activating the Erk-Smad/β-catenin signalling pathways. *Cell Biochem Funct* 2020; **38**: 87-96 [PMID: 31674048 DOI: 10.1002/cbf.3457]

48 **Garzotto CK**, Berg J, Hoffmann WE, Rand WM. Prognostic significance of serum alkaline phosphatase activity in canine appendicular osteosarcoma. *J Vet Intern Med* 2000; **14**: 587-592 [PMID: 11110379 DOI: 10.1892/0891-6640(2000)014<0587:psosap>2.3.co;2]

49 **Bacci G**, Longhi A, Versari M, Mercuri M, Briccoli A, Picci P. Prognostic factors for osteosarcoma of the extremity treated with neoadjuvant chemotherapy: 15-year experience in 789 patients treated at a single institution. *Cancer* 2006; **106**: 1154-1161 [PMID: 16421923 DOI: 10.1002/cncr.21724]

50 **Bielack SS**, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jürgens H, Winkler K. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol* 2002; **20**: 776-790 [PMID: 11821461 DOI: 10.1200/JCO.2002.20.3.776]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1116-1130

DOI: 10.4239/wjd.v12.i7.1116

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Basic Study

Expression and role of P-element-induced wimpy testis-interacting RNA in diabetic-retinopathy in mice

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Author contributions: Yu Y and Ren KM designed this study, conducted the experimental analysis, and wrote the manuscript; Chen XL revised the manuscript; all authors have read and approved the final manuscript.

Supported by National Natural Science Foundation of China, No. 81570866.

Institutional animal care and use committee statement: The study was reviewed and approved by the Institutional Review Board of Shengjing Hospital of China Medical University (Approval No. 2020PS078K).

Conflict-of-interest statement: No potential conflicts of interest relevant to this article are reported.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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Abstract

BACKGROUND

As one of the major microvascular complications of diabetes, diabetic retinopathy (DR) is the leading cause of blindness in the working age population. Because the extremely complex pathogenesis of DR has not been fully clarified, the occurrence and development of DR is closely related to tissue ischemia and hypoxia and neovascularization The formation of retinal neovascularization (RNV) has great harm to the visual acuity of patients.

AIM

To investigate the expression of P-element-induced wimpy testis-interacting RNA (piRNA) in proliferative DR mice and select piRNA related to RNV.

METHODS

One hundred healthy C57BL/6J mice were randomly divided into a normal group as control group (CG) and proliferative DR (PDR) group as experimental group (EG), with 50 mice in each group. Samples were collected from both groups at the same time, and the lesions of mice were evaluated by hematoxylin and eosin staining and retinal blood vessel staining. The retinal tissues were collected for second-generation high-throughput sequencing, and the differentially expressed piRNA between the CG and EG was detected, and polymerase chain reaction (PCR) was conducted for verification. The differentially obtained piRNA target genes and expression profiles were enrichment analysis based on gene annotation (Gene Ontology) and Kyoto Encyclopedia of Genes and Genomes.

RESULTS

In the CG there was no perfusion area, neovascularization and endothelial nucleus broke through the inner boundary membrane of retinap. In the EG, there

Manuscript source: Unsolicited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: China

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 2, 2021
Peer-review started: March 2, 2021
First decision: April 6, 2021
Revised: April 11, 2021
Accepted: May 20, 2021
Article in press: May 20, 2021
Published online: July 15, 2021

P-Reviewer: Armellini E, Fires D
S-Editor: Gao CC
L-Editor: Filipodia
P-Editor: Wang LL

were a lot of nonperfused areas, new blood vessels and endothelial nuclei breaking through the inner boundary membrane of the retina. There was a statistically significant difference in the number of vascular endothelial nuclei breaking through the inner retinal membrane between the two groups. High-throughput sequencing analysis showed that compared with the CG, a total of 79 piRNAs were differentially expressed in EG, among which 43 piRNAs were up-regulated and 36 piRNAs were down-regulated. Bioinformatics analysis showed that the differentially expressed piRNAs were mainly concentrated in the signaling pathways of angiogenesis and cell proliferation. Ten piRNAs were selected for PCR, and the results showed that the expression of piR-MMU-40373735, piR-MMU-61121420, piR-MMU-55687822, piR-MMU-1373887 were high, and the expression of piR-MMU-7401535, piR-MMU-4773779, piR-MMU-1304999, and piR-MMU-5160126 were low, which were consistent with the sequencing results.

CONCLUSION
In the EG, the abnormal expression of piRNA is involved in the pathway of angiogenesis and cell proliferation, suggesting that piRNAs have some regulatory function in proliferative diabetic-retinopathy.

Key Words: P-element-induced wimpy testis-interacting RNA; P-element-induced wimpy testis protein; High-throughput sequencing; Neovascularization; Diabetic retinopathy in mice; Bioinformatics

Core Tip: As one of the major microvascular complications of diabetes, diabetic retinopathy is the leading cause of blindness in the working age population. In the diabetic retinopathy model, the abnormal expression of P-element-induced wimpy testis-interacting RNAs (piRNAs) are involved in angiogenesis and cell proliferation pathways, suggesting that piRNAs have a certain regulatory function in diabetic retinopathy.

Citation: Yu Y, Ren KM, Chen XL. Expression and role of P-element-induced wimpy testis-interacting RNA in diabetic-retinopathy in mice. *World J Diabetes* 2021; 12(7): 1116-1130
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1116.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1116

INTRODUCTION

As one of the major microvascular complications of diabetes, diabetic retinopathy (DR) is the leading cause of blindness in working age population. Because the extremely complex pathogenesis of DR has not been fully clarified, the occurrence and development of DR is closely related to tissue ischemia and hypoxia and neovascularization[1,2]. The formation of retinal neovascularization (RNV) has great harm to the visual acuity of patients. Patients who are refractory to treatment may experience serious complications, such as neovinal glaucoma, vitreous hemorrhagia, and retinal detachment which can lead to permanent blindness. RNV is a key link in proliferative DR (PDR) and is a complex pathological process. Intravitreal injection of anti-vascular endothelial growth factor (VEGF) is an effective method for treating ocular neovascular diseases. However, the procedure can result in complications, side effects, and only short-term effects. Previous studies reported that anti-VEGF treatment cannot prevent the formation of RNV in some patients[3,4]. Therefore, it is particularly important to find other VEGF-independent pathways that promote angiogenesis to identify safe therapeutic targets against DR.

P-element-induced wimpy testis (PIWI)-interacting RNA (piRNA) was first found in the ovarian germ cells of Drosophila melanogaster in 2006. This novel short non-coding small RNA has an average length of 26-31 nucleotides[5] and functions by binding with PIWI protein to form a piRNA-induced silencing complex (PIRISC)[6-8]. piRNA plays an important role in transposon silencing, epigenetic regulation, protein

regulation of genome rearrangement, spermatogenesis, and germ stem cell maintenance[9,10]. Additionally, piRNA participates in tumor formation, human aging, and neural axon regeneration[11-13]. Based on existing studies and literature reports, which highlight the role of piRNA in neovascularization-related diseases[14, 15], piRNA may play an important role in the formation and development of DR. However, to the best of our knowledge, no previous study has explored this hypothesis. In the present study, the role of piRNA in RNV diseases was evaluated by sequencing of RNA obtained from the retinal tissues of mice with PDR mice and normal mice. The study aimed to provide theoretical support for the possible alternative clinical treatment of DR.

MATERIALS AND METHODS

Experimental animals and models

We used 100 7-d-old C57 mice of either sex in this study. All experimental animals were purchased from Changsheng Biology Co., Ltd. (Shenyang, China). The animals were fed and related operations were conducted in the animal laboratory of Shengjing Hospital in a stable, specific pathogen-free environment; the temperature was maintained at 23 ± 2 °C, with a 12 h light cycle. The experimental study was approved by the animal ethics committee of Shengjing Hospital of China Medical University (2020PS078K).

The animals were randomly divided into two groups, each consisting of 50 animals: normal group as control group (CG) and PDR group as experimental group (EG). Mice in CG were fed with mice and their mothers under normal conditions without any treatment. Briefly, 7-d-old EG mice and their mothers were housed and fed in a closed glass container with an oxygen concentration of 75% ± 2% for 5 d. Next, 12-d-old mice were fed under normal conditions, and the closed container was opened once per day to replace the bedding material, add water, and replace the mother mice[16,17]. All animals were euthanized at the age of 17 d for subsequent histopathological examination and total RNA extraction.

Retinal patch staining

Mice aged 17 d from the two groups were anesthetized and sacrificed, after which their eyeballs were removed. The eyeballs were fixed in 4% paraformaldehyde for 12 h. The contents of the anterior segment and vitreous cavity were removed, and the retina was carefully separated. The retinas were incubated in isolectin B4-594 (Invitrogen, Carlsbad, CA, United States) in a shaker at 4 °C overnight. Next, the glass slide was covered with an anti-radiation agent, and this agent was also applied to the retina[18]. The retinas were observed under a microscope (Eclipse NI, Nikon, Tokyo, Japan) and images were collected.

Hematoxylin and eosin staining

After the eyeballs of mice were removed, they were fixed in 4% paraformaldehyde for 12 h. Next, they were embedded in paraffin and cut along the sagittal plane of the optic nerve to obtain serial sections with a thickness of 4 μm. Non-continuous sections were acquired from each eye for hematoxylin and eosin (HE) staining, and sections containing the optic nerve were excluded. The number of endothelial cells breaking through the inner limiting membrane in the vitreous cavity was counted, and the average of this number was calculated in each section[19]. Only the vascular nuclei located closely to the retina were counted; thus, vascular nuclei not close to the internal limiting membrane in the vitreous cavity were excluded[20,21].

High-throughput sequencing

The total RNA of each retinal tissue sample was extracted using Trizol reagent according to the manufacturer's instructions (Takara, Japan). The quality of RNA was analyzed using NanoDrop ND-2000 (Thermo Fisher Scientific, United States). A small RNA library was constructed by real-time polymerase chain reaction (RT-PCR) using 5' and 3' linkers. Agilent 2100 and Applied Biosystems StepOnePlus Real-Time PCR systems were used to assess the quality and yield of the constructed library (Life Technologies). Finally, the RNA was sequenced by Illumina Hiseq 2000 (Illumina, San Diego, CA, United States).

Sequencing result analysis

After standardization and quality control of the sequencing data, 26-31 nt piRNAs were selected from small RNA reads, and the differential expression of piRNA was analyzed. Fold change in piRNA expression ≥ 1.5 ($P < 0.05$) was used as the threshold for determining gene upregulation or downregulation. Gene Ontology (GO) enrichment (http://www.geneontology.org/) was used to analyze the abnormal expression of genes, and KO enrichment (https://www.genome.jp/kegg/pathway) was utilized to determine the biological function of the differentially expressed piRNA and investigate its possible involvement in the disease mechanism[22].

RT-PCR

RT-PCR was performed on total RNA extracted from retina samples using Trizol reagent (Takara, Shiga, Japan) according to the manufacturer's instructions.

Statistical analysis

SPSS 22.0 software (SPSS, Inc., Chicago, IL, United States) was used for all statistical analyses. The mean ± SD of relative piRNA expression in PCR analyses was calculated. Student's *t*-test was used to analyze the difference between the two groups. $P < 0.05$ indicated a statistically significant difference.

RESULTS

Evaluation of fluorescence imaging

The samples obtained from the normal control and EGs of mice were stained with isolectin B4-594 to observe the retinal vascular structure. In the normal CG, the retinal blood vessels were intact and clear, and large blood vessels were characterized by an even radial distribution around the optic disc reaching the periphery of the retina. In the DR group, there was no perfusion in the large vessel area and no decomposition of the perfusion area. The large vessels near the optic disc were tortuous and irregular, and they were mainly visible in the middle and periphery. A large number of disordered new vessels and new vascular buds were detected at the retina boundary along with vascular leakage (Figure 1).

Quantitative analysis of retinal vascular endothelial cells

HE staining images were used to quantitatively analyze the number of retinal vascular endothelial nuclei. In the CG, the structure of the retinal internal limiting membrane was intact and smooth with occasional vascular endothelial nuclei breaking through the internal limiting membrane on the vitreous side (average number 1.163 ± 0.31). In the EG, the morphology of the internal limiting membrane was irregular, and cells under the internal limiting membrane proliferated and were arranged in a disorderly manner. A large number of clusters of vascular endothelial cells broke through the inner limiting membrane and formed the neovascular lumen (average number 29.42 ± 1.07). There was a significant difference in the number of retinal vascular endothelial cells that broke through the inner limiting membrane between the two groups ($P < 0.01$) (Figure 2).

High-throughput sequencing results

There were 79 piRNAs differentially expressed in EG compared to in CG mice, among which 43 were upregulated and 36 were downregulated (Figure 3).

Verification of results indicating differential gene expression

We selected 10 differentially expressed genes according to their gene expression levels determined by high-throughput sequencing and verified the expression levels in two groups of retina samples by RT-PCR. The primer sequences used in this study are shown in Table 1. The results of quantitative verification by RT-PCR are shown in Figure 4.

GO analysis

To further analyze the biological functions of differentially expressed genes in EG and CG mice, the GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses was performed. The results showed that the differentially expressed piRNAs were involved in many biological processes, such as sensory development, G-protein-coupled receptor signaling pathway, regulation of inflammatory factors, and visual

Table 1 Real-time polymerase chain reaction verification results of piRNA expression and list of primer sequences used

Gene ID	Fold-change	Up/down	Primer (5'-3')
piR-mmu-40373735	17.4426	Up	F: CCGGACCTCAAGCAGCC; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-61121420	17.5967	Up	F: GGCCAGCCGGGGTACA; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-55687822	17.5982	Up	F: CGCACTGCTTCACTTGACCAG; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-1373887	18.0930	Up	F: AATGATGAACCTTTTGACGGG; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-52367843	18.4806	Up	F: CGAGGACAGCCTGGTCTACACA; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-7401535	-18.5696	Down	F: CAGCCCTCGACACAAGGG; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-4773779	-18.2665	Down	F: GCACCATGATGACGGAAATT; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-1304999	-17.8006	Down	F: GCATGCAGGAGCATCAGTAGAC; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-7422255	-17.5913	Down	F: CCACATGATGATCCATAACGAGAT; R: AGTGCAGGGTCCGAGGTATT
piR-mmu-5160126	-17.4335	Down	F: GCTGAGACAGGAGGATCGCT; R: AGTGCAGGGTCCGAGGTATT

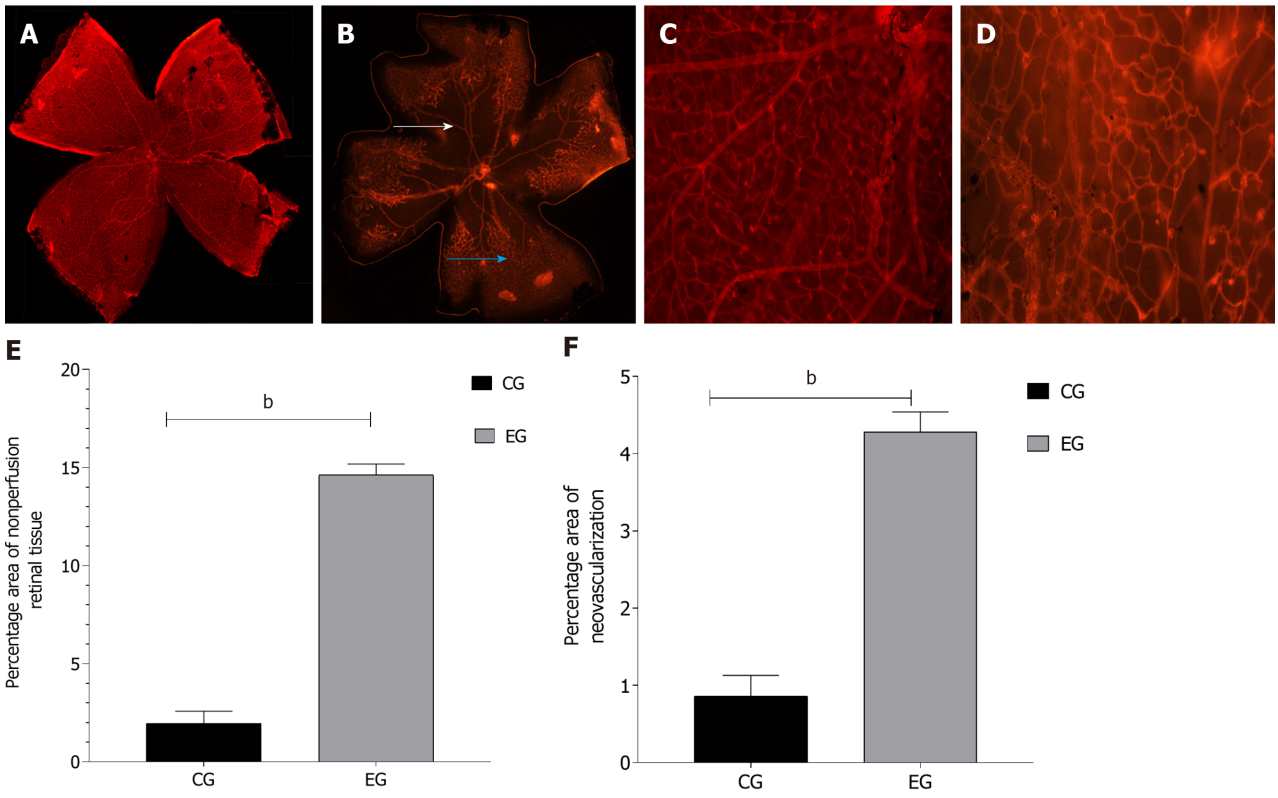


Figure 1 Evaluation of retinal neovascularization. A: Retina patch morphology of control group (CG); B: Retina tissue morphology of experimental group (EG) (white arrow indicates no perfusion area, blue arrow indicates neovascularization); C: Retina local enlarged map of CG; D: Retina local enlarged map of EG; E: Retina no perfusion area statistical map; F: Retina neovascularization cluster area statistical map. $^{b}P < 0.01$.

development, which are potentially related to RNV (Figure 5).

KEGG analysis

KEGG analysis of differentially expressed genes and proteins showed that the enriched pathways were mainly related to angiogenesis and cell proliferation and were strongly correlated with RNV (Figure 6).

Analysis of protein-protein interaction network

We obtained piRNA-target gene pairs correlated with expression level to construct the network analysis diagram. We selected the *EPO*, *HIF-1a*, *IGF1*, and *TGF-β2* genes, which are potentially related to RNV, to construct the interaction analysis diagram (Figure 7).

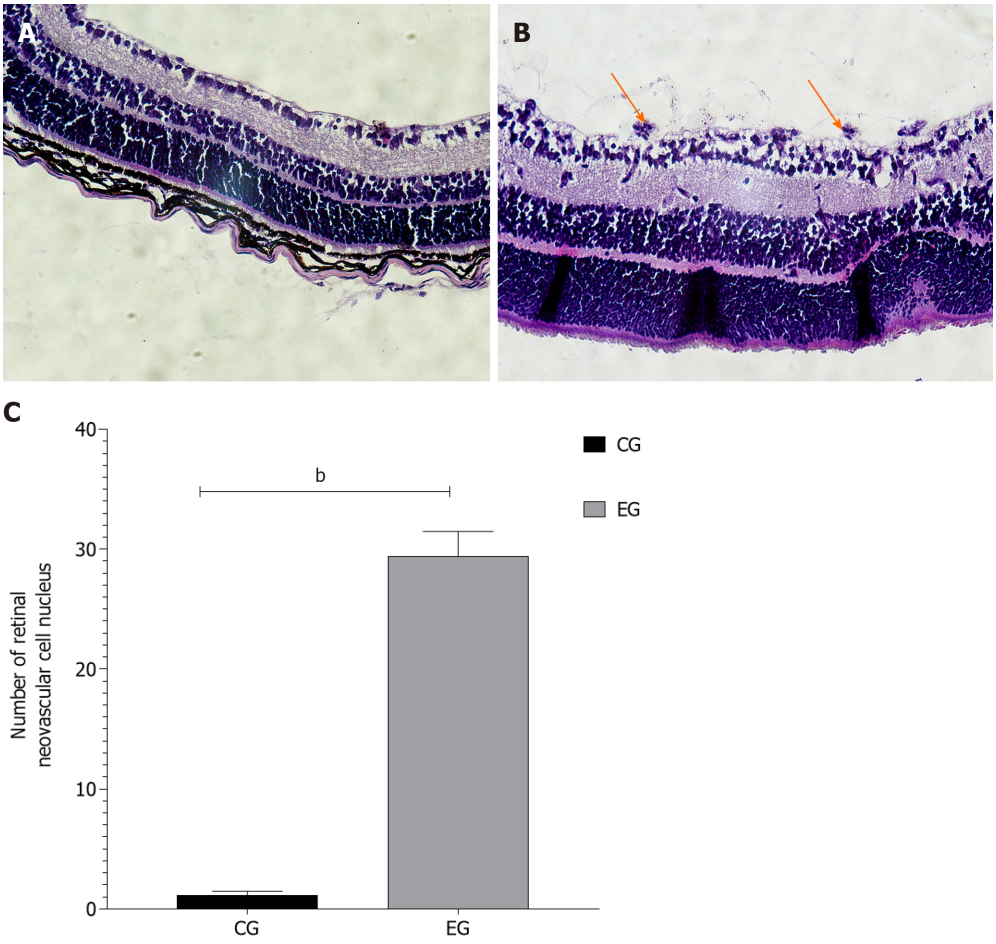


Figure 2 Quantitative analysis of vascular endothelial cells. A: Hematoxylin and eosin (HE) staining of paraffin-embedded retina sections in the control group (CG) (200 ×); B: HE staining of paraffin-embedded retina sections in the experimental group (EG) (200 ×, orange arrow indicates that endothelial cell nucleus breaks through the inner limiting membrane); C: Number of nuclei breaking through the inner limiting membrane in the two groups. $^{b}P < 0.01$.

DISCUSSION

Non-coding RNA is involved in the occurrence and development of several diseases. Numerous studies have shown that non-coding RNA is an important factor in the pathophysiological changes leading to malignant tumors and vascular diseases[23]. piRNA is a type of small non-coding RNA with an important role in animal development, reproduction, and gene regulation[10,24]. To date, 23439 piRNAs have been identified in the human genome, which is equivalent to the number of proteins encoded by mRNA (about 20000) and more than the number of miRNAs. These numbers indicate that piRNAs play an important role in regulating gene transcription [25,26].

After transcription, PIWI protein cleavage and amplification as well as piRNA clusters eventually form mature piRNAs having biological activity by forming PIRISC [27]. PIRISC, a silencing complex formed by piRNA and PIWI protein, can inhibit its target by transcriptional gene silencing and post-transcriptional gene silencing to maintain the genomic integrity of the germline[27,28]. PIRSIC regulates transposon expression at the transcriptional level by inducing epigenetic repression *via* histone H3K9me3 and DNA methylation[29]. Some studies report a larger number of allowed mismatches between target mRNA and piRNA as opposed to between target mRNA and miRNA[30]. siRNA and miRNA are easily and rapidly degraded by nucleases, whereas piRNA is relatively stable in the serum, and thus has the potential to serve as a marker for diagnosis and prediction of disease progression[31].

piRNAs are presently thought to act mainly in somatic cells and cancer tissues and are involved in cell proliferation, apoptosis, cell cycle arrest, angiogenesis, invasion, and metastasis[32]. Moreover, studies have shown that PIRISC may participate in tumorigenesis by inducing abnormal DNA methylation, which leads to genomic silencing[33]. Further, the piRNA-30473/WTAP/HK2 axis promotes the occurrence of breast cancer by regulating the methylation of m6A RNA in diffuse large B-cell

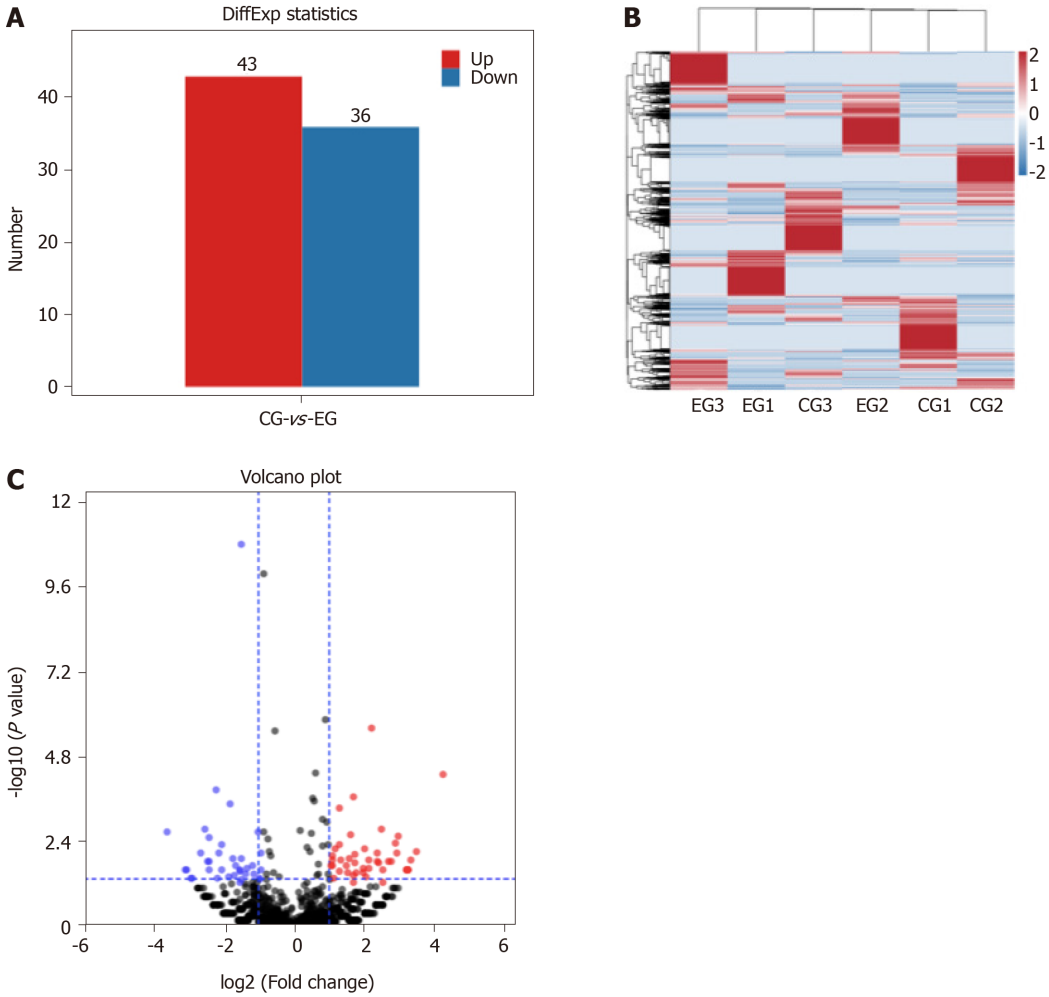


Figure 3 Screening results of differentially expressed genes. A: Number of differentially expressed piRNAs between experimental group (EG) and control group (CG); B: Clustering analysis of differentially expressed piRNAs; C: Volcano map of differentially expressed piRNAs.

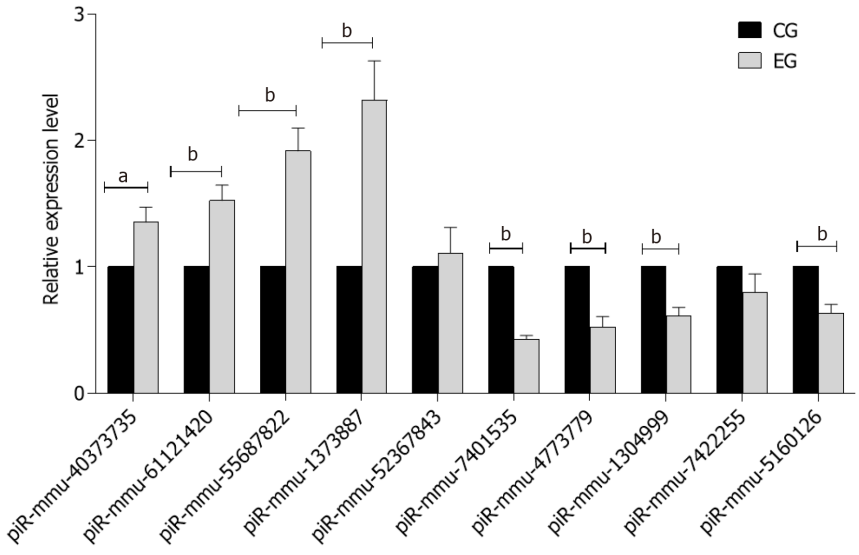
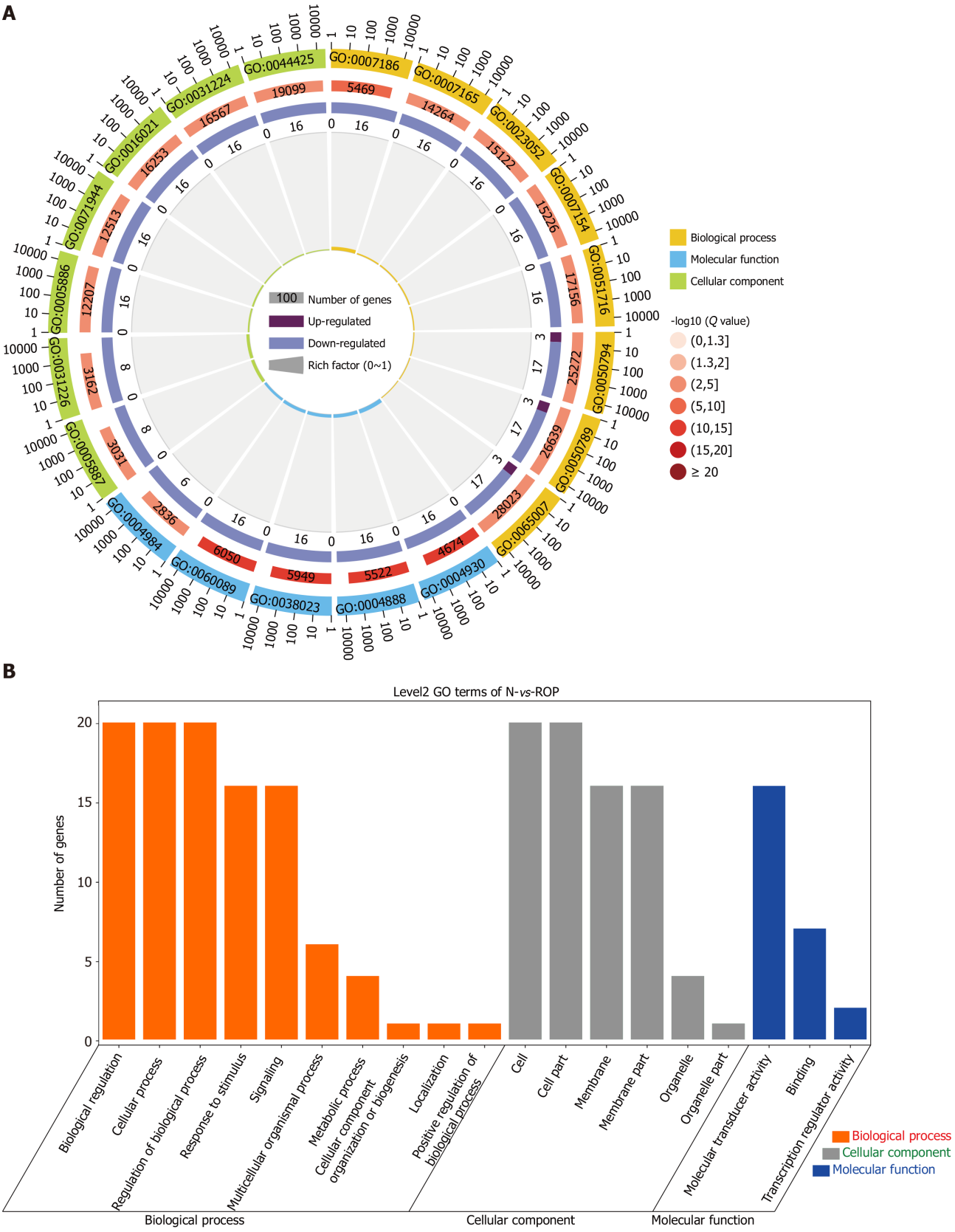


Figure 4 Statistics of ten piRNA validation results. $^{a}P < 0.05$; $^{b}P < 0.01$. CG: Control group; EG: Experimental group.

lymphoma[34]. piRNA-19166 inhibits cell migration and metastasis through the cortactin/mitochondrial membrane potential pathway in prostate cancer[35]. piRNA-823 promotes the angiogenesis of endothelial cells by promoting the secretion of VEGF and interleukin -6. It also enhances the invasion of endothelial cells by inducing the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28023
4674
5522
5949
6050
2836
3031
3162
12207
12513
16253
16567
19099
100 Number of genes
Up-regulated
Down-regulated
Rich factor (0~1)
Biological process
Molecular function
Cellular component
-log10 (Q value)
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Number of genes
Biological regulation
Cellular process
Regulation of biological process
Response to stimulus
Signaling
Multicellular organismal process
Metabolic process
Cellular component organization or biogenesis
Localization
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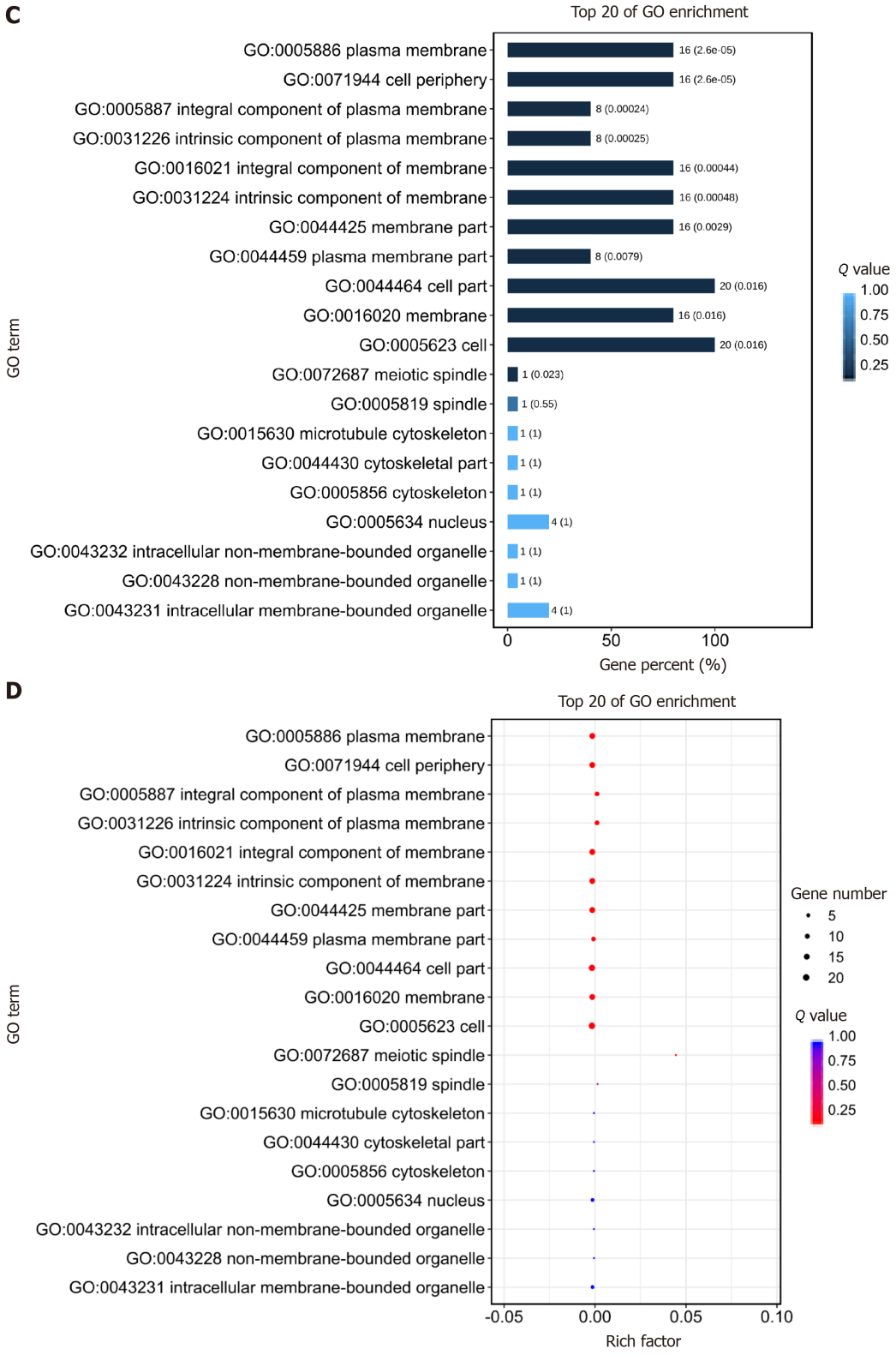

Figure 5 Gene Ontology analysis results. A: Gene Ontology (GO) enrichment circle diagram (first circle: top 20 GO terms, the coordinate scale of gene number is presented outside the circle. Second circle: number and *Q* value of the GO term in the background gene. Third circle: number of GO term piRNA target genes. Fourth circle: rich factor value of each GO term piRNA); B: Bar graph of GO enrichment (abscissa shows the level 2 GO term, whereas the ordinate shows the number of genes in the term); C: Bar graph of GO enrichment (a darker color, results in a smaller *Q* value; the value in the column is the number and *Q* value of the GO term); D: GO enrichment bubble chart (size indicates the quantity; a redder color leads to a smaller *Q* value).

expression of ICAM-1 and CXCR4[36]. Downregulation of piRNA-36712 expression is known to result in upregulation of SEPW1 expression, which in turn inhibits the expression of its downstream gene p53 and therefore it suppresses the formation of malignant tumors[37]. In addition, piRNA plays an important role in gastric cancer,

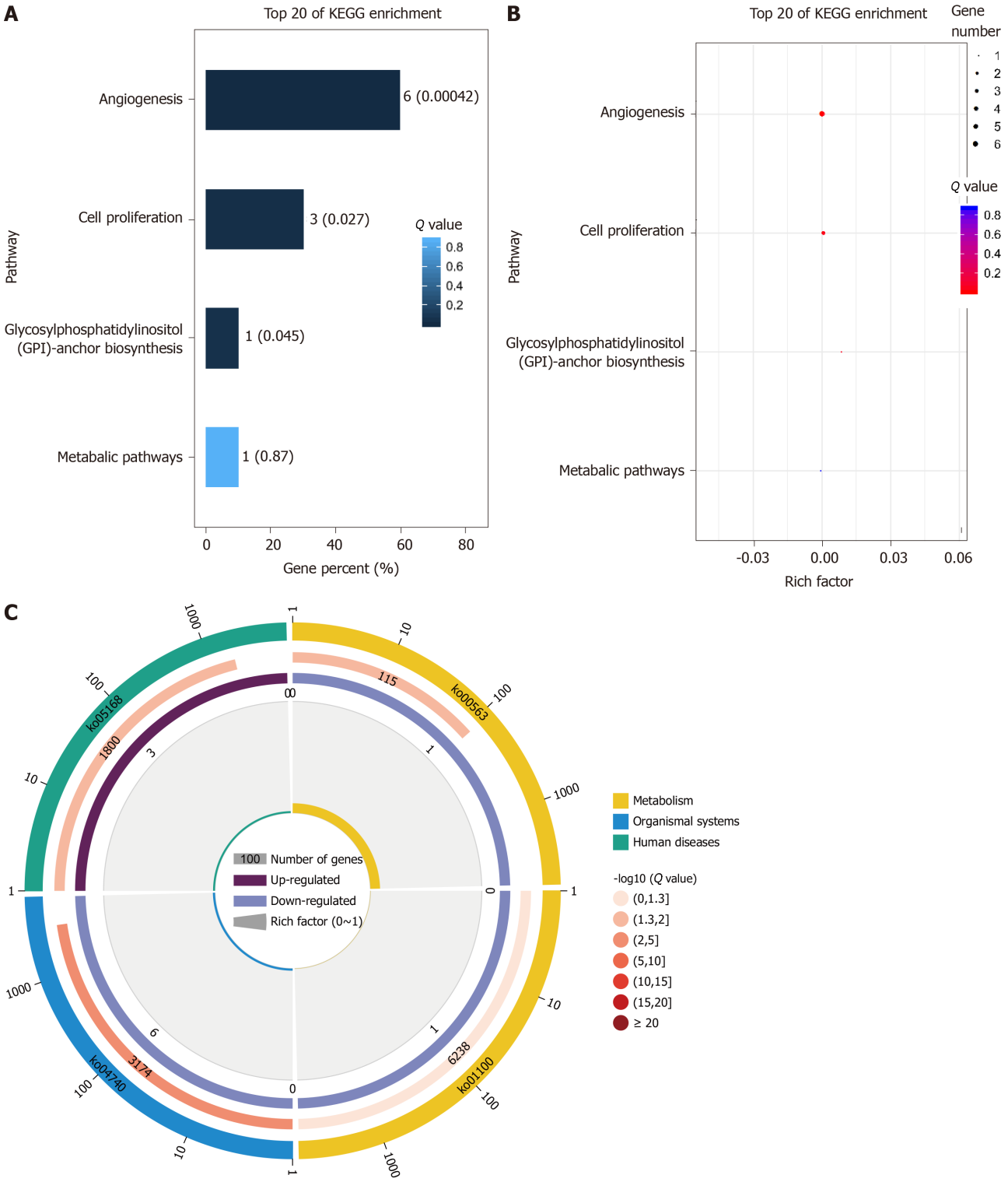

Figure 6 KO analysis results. A: KO enrichment bar chart (top 20 pathways with the lowest *Q* value were used to draw the chart. The ordinate represents the pathways, whereas the abscissa shows the percentage of the number of pathways in all piRNA target genes; a darker color correlates with a smaller the *Q* value. The value in the column is the number and *Q* value of the pathways); B: KO enrichment bubble chart [top 20 pathways with the lowest *Q* value were used to plot. The ordinate shows the pathway, whereas the abscissa shows the enrichment factor (the number of piRNA target genes in the pathway divided by all numbers). The size indicates the number; a redder color indicates a smaller *Q* value]; C: KO enrichment circle diagram [first circle: top 20 enriched pathways; outside the circle is the coordinate scale of gene number, and different colors represent different classes. Second circle: number and *Q* value of the pathway in the background gene; the more the number of genes, a longer bar and smaller *Q* value leads to a redder color. Third circle: number of piRNA target genes in the pathway. Fourth circle: rich factor value of each pathway (number of piRNA target genes in the pathway divided by all numbers); background grid line, each grid represents 0.1].

liver cancer, glioma, and other diseases. Based on these correlations, piRNA is expected to serve as a new target for cancer treatment[6,10,38].

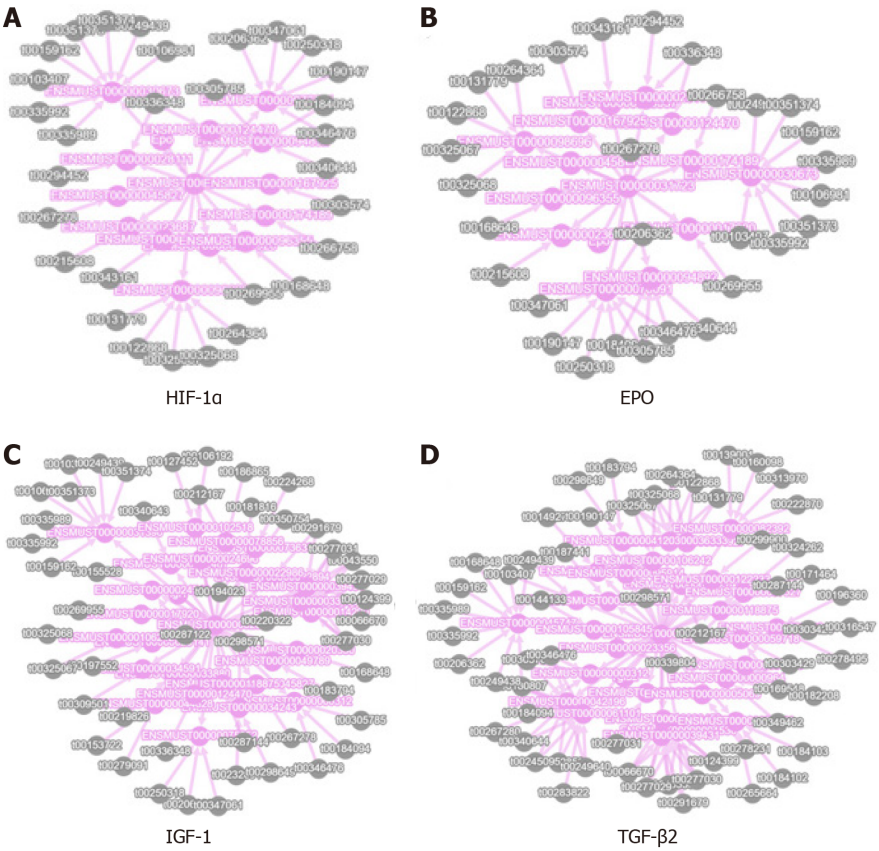

Figure 7 Protein interaction network analysis. A-D: Stand for differential expression results of piRNA and *EPO* (B), *HIF-1α* (A), *IGF1* (C), *TGF-β2* (D) which are associated with retinal neovascularization.

There are many models for the study of DR. At present, the model of RNV and vascular leakage without hyperglycemia has been applied. This model simulates PDR or the process to be developed from non-PDR (NPDR) to PDR. The retinopathy injury caused by hypoxia is due to the occurrence of the release of angiogenesis actors, presented with microhemangiomas, vascular leakage, venous occlusion, capillary in perfusion, neovascularization, and even vitreous hemorrhage, and retinal detachment [16]. There are notable nonperfusion areas in the center of the retina, the central great vessels are tortuous, and the number of vascular nuclei breaking through the inner limiting membrane is significantly increased, which are important factors indicating the success of the EG[39]. HE staining of retinal slices and paraffin sections of EG samples showed that there were large areas of nonperfusion, neovascularization, and vascular endothelial cells breaking through the inner limiting membrane in the retina.

DR is one of the most common microvascular diseases of diabetes and also the main cause of blindness in diabetic patients[40]. Studies have shown that DR occurs in both type 1 diabetes and type 2 diabetes[41]. With the increase in the number of diabetic patients, DR has become the main cause of visual impairment in diabetic patients[42]. The whole pathological process of DR includes important pathological changes such as loss of retinal capillary pericytes, thickening of basement membrane, loss of endothelial barrier function, destruction of blood-retinal barrier, and lead to retinal ischemia, which will increase the level of VEGF. Studies have shown that overexpression of VEGF is associated with RNV, which can cause retinal hemorrhage, macular edema, retinal detachment, and neovascularization glaucoma, *etc.*, leading to severe visual impairment and eventually blindness.

The development of DR involves two stages: early NPDR and advanced PDR. The former is mainly characterized by increased retinal permeability and intraretinal hemorrhage, while the latter is mainly manifested by RNV. In NPDR, high glucose induced retinopathy mainly includes loss of capillary pericytes, thinning of the vascular layer and destruction of the blood-retinal barrier, which further leads to retinal ischemia and hypoxia. When the disease progresses to PDR, neovascularization occurs and eventually leads to severe visual impairment. RNV is a common pathological change in many retinopathies, including DR, retinopathy of prematurity, and age-related macular degeneration.

The common feature of clinical treatment of RNV as well as malignant tumors is treatment with targeted drugs, mainly anti-VEGF drugs. However, targeted drugs against RNV have short action time and require multiple intraocular injections, creating safety risks. Based on of the findings of previous studies on the role of piRNA in cancers and neovascularization-related diseases, we compared piRNA expression levels between the DR model and CGs. The results revealed 79 piRNAs with differential expression in EG and CG. Through GO and KEGG analysis, we established that the mRNA of the differentially expressed piRNAs was involved in processes such as angiogenesis, optic nerve development, inflammation, and proliferation of cells. Among them, *EPO*, *HIF-1a*, *IGF1*, *TGF-β2*, and other genes are closely related to angiogenesis. Interestingly, a change in *EPO* expression is considered as an important factor affecting the retinopathy of prematurity. *EPO* treatment can effectively protect the nervous system and optic nerve development of premature infants[42,43]. *HIF-1a* is stably expressed under hypoxia; it can regulate *EPO* and VEGF expression and promote RNV[44,45]. Inhibiting the VEGF/VEGFR2 and HIF-1α/VEGF signaling pathways can prevent angiogenesis[46]. TGF-β2 is a pro-inflammatory cytokine precursor related to the pathogenesis of DR. IGF1 has been identified as the direct target of miR-142-5p. It can reduce the level of miR-142-5p by activating the IGF1/IGF1R median signaling pathway (involving p-PI3K, p-ERK, p-Akt, and VEGF activation), eventually leading to cell proliferation and is involved in the pathological process of DR[47,48].

piRNA plays an important role in various diseases. By interacting with PIWI protein, piRNA can participate in cancer formation and neovascularization through DNA methylation. It can also affect the expression of target genes. We examined the differential expression of piRNA in an oxygen-induced retinopathy mouse model and the potential cellular pathways involved in this process. The study identified a set of target genes that can enhance the theoretical understanding of the role of piRNA in RNV. Because the specific mechanism of action has not been studied in detail, studies are needed to explore its mechanism in a larger sample size. Moreover, it can drive further research on new strategies for clinical treatment. We plan to predict the downstream targets of each differentially expressed piRNA and verify the predicted targets using molecular biology methods. These results provide a foundation for further exploration of the molecular mechanism underlying the development of PDR.

CONCLUSION

Abnormal expression of the piRNAs are involved in pathways of angiogenesis and cell proliferation, which suggests that piRNAs may regulate some functions in proliferative DR.

ARTICLE HIGHLIGHTS

Research background

Retinal neovascularization is caused by the progression of ischemic retinal diseases, including diabetic retinopathy, retinopathy of prematurity, age-related macular degeneration, and retinal vein occlusion. Complications of retinal neovascularization can severely impair vision or lead to permanent blindness.

Research motivation

To explore the upstream molecules of vascular endothelial growth factor or rate-limiting steps of angiogenesis, and to reveal new approaches to the treatment of diabetic retinal.

Research objectives

The research on the role of piRNAs (p-Element-induced wimpy testis-interacting RNAs) in retinal neovascularization disease is expected to provide theoretical support for the clinical treatment of diabetic retina.

Research methods

A diabetic retinopathy model was established. The differentially expressed piRNA

was screened by high-throughput sequencing, and the differentially expressed piRNA was selected according to the sequencing results, and verified by polymerase chain reaction.

Research results

A total of 79 piRNAs were differentially expressed in experimental group, of which 43 were upregulated and 36 were down-regulated.

Research conclusions

piRNAs were differentially expressed in DR model. Differentially expressed piRNAs is involved in the formation of retinal neovascularization. Differentially expressed piRNAs can regulate retinal development and retinal angiopathy through a variety of signaling pathways.

Research perspectives

Drugs targeting piRNAs may be novel candidates for the treatment of diabetic retinopathy.

REFERENCES

1 **Hou L**, Shi Y, Wang S, Chen Q, Li Q, Zhao M, Zhou X. Associations of serum uric acid level with diabetic retinopathy and albuminuria in patients with type 2 diabetes mellitus. *J Int Med Res* 2020; **48**: 300060520963980 [PMID: 33334216 DOI: 10.1177/0300060520963980]

2 **Park W**, Kim J, Choi S, Kim T, Park M, Kim S, You JC, Kim JH, Ha KS, Lee JH, Kwon YG, Kim YM. Human plasminogen-derived N-acetyl-Arg-Leu-Tyr-Glu antagonizes VEGFR-2 to prevent blood-retinal barrier breakdown in diabetic mice. *Biomed Pharmacother* 2021; **134**: 111110 [PMID: 33338749 DOI: 10.1016/j.biopha.2020.111110]

3 **Delu S**, Peiying H, Brian L V, Joshua L D, Juan E G, Ebenezer D, Maureen G M, Daniel F M, Gui-Shuang Y; and the CATT Research Group. Systemic Medication Use and the Incidence and Growth of Geographic Atrophy in the Comparison of Age-related Macular Degeneration Treatments Trials (CATT). *Retina* 2021; **41**: 1455-1462 [PMID: 33332813 DOI: 10.1097/IAE.0000000000003075]

4 **Infeld DA**, O'Shea JG. Diabetic retinopathy. *Postgrad Med J* 1998; **74**: 129-133 [PMID: 9640436 DOI: 10.1136/pgmj.74.869.129]

5 **Girard A**, Sachidanandam R, Hannon GJ, Carmell MA. A germline-specific class of small RNAs binds mammalian Piwi proteins. *Nature* 2006; **442**: 199-202 [PMID: 16751776 DOI: 10.1038/nature04917]

6 **Tamtaji OR**, Behnam M, Pourattar MA, Hamblin MR, Mahjoubin-Tehran M, Mirzaei H, Asemi Z. PIWI-interacting RNAs and PIWI proteins in glioma: molecular pathogenesis and role as biomarkers. *Cell Commun Signal* 2020; **18**: 168 [PMID: 33109195 DOI: 10.1186/s12964-020-00657-z]

7 **Vinasco-Sandoval T**, Moreira FC, F Vidal A, Pinto P, Ribeiro-Dos-Santos AM, Cruz RLS, Fonseca Cabral G, Anaissi AKM, Lopes KP, Ribeiro-Dos-Santos A, Demachki S, de Assumpção PP, Ribeiro-Dos-Santos Â, Santos S. Global Analyses of Expressed Piwi-Interacting RNAs in Gastric Cancer. *Int J Mol Sci* 2020; **21** [PMID: 33081152 DOI: 10.3390/ijms21207656]

8 **Zou GL**, Zhang XR, Ma YL, Lu Q, Zhao R, Zhu YZ, Wang YY. The role of Nrf2/PIWIL2/purine metabolism axis in controlling radiation-induced lung fibrosis. *Am J Cancer Res* 2020; **10**: 2752-2767 [PMID: 33042615]

9 **Rayford KJ**, Cooley A, Arun A, Rachakonda G, Kleschenko Y, Villalta F, Pratap S, Lima MF, Nde PN. *Trypanosoma cruzi* Modulates PIWI-Interacting RNA Expression in Primary Human Cardiac Myocytes during the Early Phase of Infection. *Int J Mol Sci* 2020; **21** [PMID: 33322418 DOI: 10.3390/ijms21249439]

10 **Beltran T**, Pahita E, Ghosh S, Lenhard B, Sarkies P. Integrator is recruited to promoter-proximally paused RNA Pol II to generate Caenorhabditis elegans piRNA precursors. *EMBO J* 2021; **40**: e105564 [PMID: 33340372 DOI: 10.15252/embj.2020105564]

11 **Toden S**, Zumwalt TJ, Goel A. Non-coding RNAs and potential therapeutic targeting in cancer. *Biochim Biophys Acta Rev Cancer* 2021; **1875**: 188491 [PMID: 33316377 DOI: 10.1016/j.bbcan.2020.188491]

12 **Onishi R**, Sato K, Murano K, Negishi L, Siomi H, Siomi MC. Piwi suppresses transcription of Brahma-dependent transposons *via* Maelstrom in ovarian somatic cells. *Sci Adv* 2020; **6** [PMID: 33310860 DOI: 10.1126/sciadv.aaz7420]

13 **Lee YJ**, Moon SU, Park MG, Jung WY, Park YK, Song SK, Ryu JG, Lee YS, Heo HJ, Gu HN, Cho SJ, Ali BA, Al-Khedhairy AA, Lee I, Kim S. Multiplex bioimaging of piRNA molecular pathway-regulated theragnostic effects in a single breast cancer cell using a piRNA molecular beacon. *Biomaterials* 2016; **101**: 143-155 [PMID: 27289065 DOI: 10.1016/j.biomaterials.2016.05.052]

14 **Supe S**, Upadhya A, Singh K. Role of small interfering RNA (siRNA) in targeting ocular neovascularization: A review. *Exp Eye Res* 2021; **202**: 108329 [PMID: 33198953 DOI: 10.1016/j.exer.2020.108329]

15 **Masjedi A**, Ahmadi A, Atyabi F, Farhadi S, Irandoust M, Khazaei-Poul Y, Ghasemi Chaleshtari M, Edalati Fathabad M, Baghaei M, Haghnavaz N, Baradaran B, Hojjat-Farsangi M, Ghalamfarsa G, Sabz G, Hasanzadeh S, Jadidi-Niaragh F. Silencing of IL-6 and STAT3 by siRNA loaded hyaluronate-N,N,N-trimethyl chitosan nanoparticles potently reduces cancer cell progression. *Int J Biol Macromol* 2020; **149**: 487-500 [PMID: 32004600 DOI: 10.1016/j.ijbiomac.2020.01.273]

16 **Olivares AM**, Althoff K, Chen GF, Wu S, Morrisson MA, DeAngelis MM, Haider N. Animal Models of Diabetic Retinopathy. *Curr Diab Rep* 2017; **17**: 93 [PMID: 28836097 DOI: 10.1007/s11892-017-0913-0]

17 **Cheung N**, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet* 2010; **376**: 124-136 [PMID: 20580421 DOI: 10.1016/S0140-6736(09)62124-3]

18 **Geng W**, Qin F, Ren J, Xiao S, Wang A. Mini-peptide RPL41 attenuated retinal neovascularization by inducing degradation of ATF4 in oxygen-induced retinopathy mice. *Exp Cell Res* 2018; **369**: 243-250 [PMID: 29803741 DOI: 10.1016/j.yexcr.2018.05.027]

19 **Wang Y**, Wang X, Ma Y, Wang YX, Di Y. Expression profiles of long noncoding RNAs in retinopathy of prematurity. *Neural Regen Res* 2020; **15**: 1962-1968 [PMID: 32246647 DOI: 10.4103/1673-5374.280328]

20 **Pelikánová T**. [Diabetic retinopathy: pathogenesis and therapeutic implications]. *Vnitr Lek***62**: 620-628 [PMID: 27627088]

21 **Scott A**, Powner MB, Fruttiger M. Quantification of vascular tortuosity as an early outcome measure in oxygen induced retinopathy (OIR). *Exp Eye Res* 2014; **120**: 55-60 [PMID: 24418725 DOI: 10.1016/j.exer.2013.12.020]

22 **Li Y**, Min Cheng, Zhao Y, Wang J. Effects of fluoride on PIWI-interacting RNA expression profiling in testis of mice. *Chemosphere* 2021; **269**: 128727 [PMID: 33213873 DOI: 10.1016/j.chemosphere.2020.128727]

23 **Kumar S**, Gonzalez EA, Rameshwar P, Etchegaray JP. Non-Coding RNAs as Mediators of Epigenetic Changes in Malignancies. *Cancers (Basel)* 2020; **12** [PMID: 33291485 DOI: 10.3390/cancers12123657]

24 **Xin J**, Du M, Jiang X, Wu Y, Ben S, Zheng R, Chu H, Li S, Zhang Z, Wang M. Systematic evaluation of the effects of genetic variants on PIWI-interacting RNA expression across 33 cancer types. *Nucleic Acids Res* 2021; **49**: 90-97 [PMID: 33330918 DOI: 10.1093/nar/gkaa1190]

25 **Martinez VD**, Vucic EA, Thu KL, Hubaux R, Enfield KS, Pikor LA, Becker-Santos DD, Brown CJ, Lam S, Lam WL. Unique somatic and malignant expression patterns implicate PIWI-interacting RNAs in cancer-type specific biology. *Sci Rep* 2015; **5**: 10423 [PMID: 26013764 DOI: 10.1038/srep10423]

26 **Moyano M**, Stefani G. piRNA involvement in genome stability and human cancer. *J Hematol Oncol* 2015; **8**: 38 [PMID: 25895683 DOI: 10.1186/s13045-015-0133-5]

27 **Czech B**, Hannon GJ. One Loop to Rule Them All: The Ping-Pong Cycle and piRNA-Guided Silencing. *Trends Biochem Sci* 2016; **41**: 324-337 [PMID: 26810602 DOI: 10.1016/j.tibs.2015.12.008]

28 **Iwasaki YW**, Siomi MC, Siomi H. PIWI-Interacting RNA: Its Biogenesis and Functions. *Annu Rev Biochem* 2015; **84**: 405-433 [PMID: 25747396 DOI: 10.1146/annurev-biochem-060614-034258]

29 **Luteijn MJ**, Ketting RF. PIWI-interacting RNAs: from generation to transgenerational epigenetics. *Nat Rev Genet* 2013; **14**: 523-534 [PMID: 23797853 DOI: 10.1038/nrg3495]

30 **Watanabe T**, Lin H. Posttranscriptional regulation of gene expression by Piwi proteins and piRNAs. *Mol Cell* 2014; **56**: 18-27 [PMID: 25280102 DOI: 10.1016/j.molcel.2014.09.012]

31 **Balaratnam S**, West N, Basu S. A piRNA utilizes HILI and HIWI2 mediated pathway to down-regulate ferritin heavy chain 1 mRNA in human somatic cells. *Nucleic Acids Res* 2018; **46**: 10635-10648 [PMID: 30102404 DOI: 10.1093/nar/gky728]

32 **Lin Y**, Zheng J, Lin D. PIWI-interacting RNAs in human cancer. *Semin Cancer Biol* 2020 [PMID: 32877760 DOI: 10.1016/j.semcancer.2020.08.012]

33 **Siddiqi S**, Matushansky I. Piwis and piwi-interacting RNAs in the epigenetics of cancer. *J Cell Biochem* 2012; **113**: 373-380 [PMID: 21928326 DOI: 10.1002/jcb.23363]

34 **Han H**, Fan G, Song S, Jiang Y, Qian C, Zhang W, Su Q, Xue X, Zhuang W, Li B. piRNA-30473 contributes to tumorigenesis and poor prognosis by regulating m6A RNA methylation in DLBCL. *Blood* 2021; **137**: 1603-1614 [PMID: 32967010 DOI: 10.1182/blood.2019003764]

35 **Qi T**, Cao H, Sun H, Feng H, Li N, Wang C, Wang L. piR-19166 inhibits migration and metastasis through CTTN/MMPs pathway in prostate carcinoma. *Aging (Albany NY)* 2020; **12**: 18209-18220 [PMID: 32881713 DOI: 10.18632/aging.103677]

36 **Yan H**, Wu QL, Sun CY, Ai LS, Deng J, Zhang L, Chen L, Chu ZB, Tang B, Wang K, Wu XF, Xu J, Hu Y. piRNA-823 contributes to tumorigenesis by regulating de novo DNA methylation and angiogenesis in multiple myeloma. *Leukemia* 2015; **29**: 196-206 [PMID: 24732595 DOI: 10.1038/leu.2014.135]

37 **Tan L**, Mai D, Zhang B, Jiang X, Zhang J, Bai R, Ye Y, Li M, Pan L, Su J, Zheng Y, Liu Z, Zuo Z, Zhao Q, Li X, Huang X, Yang J, Tan W, Zheng J, Lin D. PIWI-interacting RNA-36712 restrains breast cancer progression and chemoresistance by interaction with SEPW1 pseudogene SEPW1P RNA. *Mol Cancer* 2019; **18**: 9 [PMID: 30636640 DOI: 10.1186/s12943-019-0940-3]

38 **Zhou J**, Zhou W, Zhang R. The potential mechanisms of piRNA to induce hepatocellular carcinoma in human. *Med Hypotheses* 2021; **146**: 110400 [PMID: 33229192 DOI: 10.1016/j.mehy.2020.110400]

39 **Solomon SD**, Chew E, Duh EJ, Sobrin L, Sun JK, VanderBeek BL, Wykoff CC, Gardner TW.

Diabetic Retinopathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 412-418 [PMID: 28223445 DOI: 10.2337/dc16-2641]

40 **Recchia FM**, Xu L, Penn JS, Boone B, Dexheimer PJ. Identification of genes and pathways involved in retinal neovascularization by microarray analysis of two animal models of retinal angiogenesis. *Invest Ophthalmol Vis Sci* 2010; **51**: 1098-1105 [PMID: 19834031 DOI: 10.1167/iovs.09-4006]

41 **Yoshida S**, Nakama T, Ishikawa K, Arima M, Tachibana T, Nakao S, Sassa Y, Yasuda M, Enaida H, Oshima Y, Kono T, Ishibashi T. Antiangiogenic shift in vitreous after vitrectomy in patients with proliferative diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2012; **53**: 6997-7003 [PMID: 22977138 DOI: 10.1167/iovs.12-9671]

42 **Natalucci G**, Latal B, Koller B, Rüegger C, Sick B, Held L, Fauchère JC; Swiss EPO Neuroprotection Trial Group. Neurodevelopmental Outcomes at Age 5 Years After Prophylactic Early High-Dose Recombinant Human Erythropoietin for Neuroprotection in Very Preterm Infants. *JAMA* 2020; **324**: 2324-2327 [PMID: 33289818 DOI: 10.1001/jama.2020.19395]

43 **Arellano-Buendía AS**, Castañeda-Lara LG, Loredo-Mendoza ML, García-Arroyo FE, Rojas-Morales P, Argüello-García R, Juárez-Rojas JG, Tapia E, Pedraza-Chaverri J, Sánchez-Lozada LG, Osorio-Alonso H. Effects of Allicin on Pathophysiological Mechanisms during the Progression of Nephropathy Associated to Diabetes. *Antioxidants (Basel)* 2020; **9** [PMID: 33203103 DOI: 10.3390/antiox9111134]

44 **Wang H**, Xu X, Yin Y, Yu S, Ren H, Xue Q. Catalpol protects vascular structure and promotes angiogenesis in cerebral ischemic rats by targeting HIF-1α/VEGF. *Phytomedicine* 2020; **78**: 153300 [PMID: 32866905 DOI: 10.1016/j.phymed.2020.153300]

45 **Lazzara F**, Trotta MC, Platania CBM, D'Amico M, Petrillo F, Galdiero M, Gesualdo C, Rossi S, Drago F, Bucolo C. Stabilization of HIF-1α in Human Retinal Endothelial Cells Modulates Expression of miRNAs and Proangiogenic Growth Factors. *Front Pharmacol* 2020; **11**: 1063 [PMID: 32848728 DOI: 10.3389/fphar.2020.01063]

46 **Han JM**, Choi YS, Dhakal D, Sohng JK, Jung HJ. Novel Nargenicin A1 Analog Inhibits Angiogenesis by Downregulating the Endothelial VEGF/VEGFR2 Signaling and Tumoral HIF-1α/VEGF Pathway. *Biomedicines* 2020; **8** [PMID: 32751120 DOI: 10.3390/biomedicines8080252]

47 **Bonfiglio V**, Platania CBM, Lazzara F, Conti F, Pizzo C, Reibaldi M, Russo A, Fallico M, Ortisi E, Pignatelli F, Longo A, Avitabile T, Drago F, Bucolo C. TGF-β Serum Levels in Diabetic Retinopathy Patients and the Role of Anti-VEGF Therapy. *Int J Mol Sci* 2020; **21** [PMID: 33334029 DOI: 10.3390/ijms21249558]

48 **Liu X**, Li J, Li X. miR-142-5p regulates the progression of diabetic retinopathy by targeting IGF1. *Int J Immunopathol Pharmacol* 2020; **34**: 2058738420909041 [PMID: 32116075 DOI: 10.1177/2058738420909041]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 July 15; 12(7): 1131-1140

DOI: 10.4239/wjd.v12.i7.1131

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Observational Study

Fasting biochemical hypoglycemia and related-factors in non-diabetic population: Kanagawa Investigation of Total Check-up Data from National Database-8

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Author contributions: Nakajima K and Nakamura T conceived and designed original study; Tanaka K and Nakajima K analyzed the data; Higuchi R and Mizusawa K prepared the documents related to the study and organized the data; Tanaka K and Nakajima K wrote the first draft of the manuscript.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of Kanagawa University of Human Services Institutional Review Board.

Informed consent statement: Informed written consent was waived.

Conflict-of-interest statement: The authors declare that they have no conflict-of-interest.

Data sharing statement: Not available.

STROBE statement: The authors

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Abstract

BACKGROUND

In healthy people, the lowest daily blood glucose concentration is usually observed in the early morning, after overnight fasting. However, the clinical relevance and the prevalence of fasting biochemical hypoglycemia (FBH) are poorly understood in people who do not have diabetes, although the clinical implications of such hypoglycemia have been extensively studied in patients with diabetes. FBH can be influenced by many factors, including age, sex, body mass, smoking, alcohol drinking, exercise levels, medications, and eating behaviors, such as breakfast skipping and late-night eating.

AIM

To determine the prevalence of FBH and investigated its association with potential risk factors in a population without diabetes.

METHODS

Clinical parameters and lifestyle-related factors were assessed in a cross-sectional study of 695613 people aged 40-74 years who had undergone a health check-up (390282 men and 305331 women). FBH was defined as fasting plasma glucose < 70 mg/dL (3.9 mmol/L) after overnight fasting, regardless of any symptoms. The absence of diabetes was defined as HbA1c < 6.5%, fasting plasma glucose < 126 mg/dL (7.0 mmol/L), and no pharmacotherapy for diabetes. Multivariate logistic

have read the STROBE statement, and the manuscript was prepared and revised according to the STROBE statement.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Japan

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 8, 2021
Peer-review started: February 8, 2021
First decision: April 20, 2021
Revised: April 28, 2021
Accepted: June 22, 2021
Article in press: June 22, 2021
Published online: July 15, 2021

P-Reviewer: Ng SM
S-Editor: Zhang H
L-Editor: A
P-Editor: Wang LL

regression analysis, with adjustment for confounding factors, was used to identify associations.

RESULTS

FBH was present in 1842 participants (0.26%). There were significantly more women in the FBH group (59.1%) than in the non-FBH group (43.9%). Values of most of the clinical parameters, but not age, were significantly lower in the FBH group than in the non-FBH group. Logistic regression analysis showed that a body mass index of ≤ 20.9 kg/m^2 (reference: 21-22.9 kg/m^2) and current smoking were significantly associated with FBH, and this was not altered by adjustment for age, sex, and pharmacotherapy for hypertension or dyslipidemia. Female sex was associated with FBH. When the data were analyzed according to sex, men in their 60s or 70s appeared more likely to experience FBH compared with their 40s, whereas men in their 50s and women aged ≥ 50 years appeared less likely to experience FBH. The relationships of FBH with other factors including alcohol drinking and pharmacotherapies for hypertension and dyslipidemia also differed between men and women.

CONCLUSION

FBH occurs even in non-diabetic people, albeit at a very low frequency. FBH is robustly associated with low body mass and smoking, and its relationship with lifestyle factors varies according to sex.

Key Words: Hypoglycemia; Body mass index; Age; Smoking; Women; Breakfast skipping

Core Tip: The clinical relevance of fasting biochemical hypoglycemia (FBH) is poorly understood in people who do not have diabetes. Therefore, we determined the prevalence of FBH and its relationships with other parameters in approximately 700000 people who did not have diabetes. FBH was identified in 0.26% of the participants and women were over-represented among these (59.1%). Low body mass and smoking were associated with FBH in both men and women. Women and men in their 60s and 70s were more likely to experience FBH, and the relationships of FBH with other factors differed between men and women.

Citation: Tanaka K, Higuchi R, Mizusawa K, Nakamura T, Nakajima K. Fasting biochemical hypoglycemia and related-factors in non-diabetic population: Kanagawa Investigation of Total Check-up Data from National Database-8. *World J Diabetes* 2021; 12(7): 1131-1140
URL: https://www.wjgnet.com/1948-9358/full/v12/i7/1131.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i7.1131

INTRODUCTION

Hypoglycemia is one of the most serious complications during the treatment of diabetes, whether it be type 1, type 2, or another type[1-4]. Hypoglycemia is associated with high mortality due to cardiovascular events and impaired cognitive function. The prevalence and severity of hypoglycemia, which is particularly frequent in diabetic patients who are administering insulin[1-4], are affected by multiple factors, including medication, diet, exercise, and the presence of comorbidities. However, the clinical relevance of hypoglycemia is poorly understood in people who do not have diabetes, and its prevalence has been determined in only a few small studies[5-7]. In apparently healthy people, blood glucose concentration tends to be lowest under fasting conditions in the early morning, after overnight fasting.

We therefore aimed to determine the prevalence of fasting biochemical hypoglycemia (FBH), which was assessed using a sample of plasma instead of finger-stick blood, and to identify the associated factors from among age, sex, body mass, smoking, alcohol drinking, exercise status, and breakfast skipping in a general population of people without diabetes, using healthcare data provided by the Japanese

Ministry of Health, Labour, and Welfare.

MATERIALS AND METHODS

Study design and participants

The overarching study was a composite multidisciplinary study that consisted of the secondary use of annual health check-up data collected in Japan (Kanagawa Investigation of the Total Check-up Data from the National Database) that aimed to determine the clinical factors associated with cardiometabolic diseases. Details of the study concept and design have been published elsewhere[8]. The present study was performed using data from individuals who underwent specific health check-ups and were living in Kanagawa Prefecture between April 2012 and March 2013. The study protocol was approved by the Ethics Committee of Kanagawa University of Human Services (10-43) and the Ministry of Health, Labour, and Welfare of Japan (No. 121).

We received digitally recorded anonymous data from the Ministry of Health, Labour, and Welfare of Japan in 2017, as part of its nationwide program for the provision of medical data to third parties[9]. To protect against the identification of specific individuals, their ages had been categorized as 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, or 70-74 years. However, for the purposes of the present study, to evaluate age as a single numeric value, we transformed the age groups into substituted ages (st-age), corresponding to the median for each age group (42, 47, 52, 57, 62, 67, and 72 years, respectively).

We initially reviewed data collected from 1623399 non-hospitalized people aged 40-74 years who had attended health check-ups. As shown in Figure 1, individuals who were undergoing pharmacotherapy for diabetes and/or had an HbA1c of ≥ 6.5%, regardless of their type of diabetes, were excluded (n = 95664; 5.9%). Individuals with a fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L) were also excluded (n = 34336). After the further exclusion of those for whom incomplete clinical and lifestyle data were available (n = 797786), data from 695613 individuals remained for analysis (390282 men and 305331 women).

Measurements

Anthropometric and laboratory measurements were performed in the morning following an overnight fast. Overnight fasting for > 10 h was individually confirmed and recorded by a staff member[8]. Body mass, waist circumference at the level of the navel, and height were measured by trained staff members. Body mass index (BMI) was calculated as mass (kg) divided by the square of height (m^2). The participants were then placed into eight BMI groups: ≤ 16.9, 17-18.9, 19-20.9, 21-22.9, 23-24.9, 25-26.9, 27-28.9, and ≥ 29.0 kg/m^2.

Fasting plasma glucose was measured (approximately 54% of samples) mainly by spectrophotometric or potentiometric (approximately 30%) method[8]. FBH was defined as an FPG < 70 mg/dL (3.9 mmol/L), regardless of the presence or absence of symptoms[4,10]. Laboratory measurements were performed using standard automated methods. Habitual breakfast skipping was defined as a positive response to the question: "Do you skip breakfast at least three times per week?"[8].

Biostatistics

Data are expressed as mean ± SD or medians (interquartile range). Differences in continuous and categorical datasets between FBH and non-FBH groups were evaluated using Student's t-test or the χ^2 test, respectively. Trends in the prevalence of FBH with increasing alcohol consumption and age (three groups were defined: people in their 40s, 50s, and 60s + 70s) were evaluated using Cochran-Armitage tests. Because the number of participants in their 70s was relatively low, those in their 70s were grouped with those in their 60s for the analysis.

A logistic regression model was used to evaluate the relationships between FBH and plausibly related factors, with adjustment for potential confounding factors (age, sex, pharmacotherapy for hypertension or dyslipidemia, smoking status, alcohol consumption, and exercise status), yielding adjusted odds ratios (ORs) and 95%CIs. As in our previous study[11], a BMI range of 21.0-22.9 kg/m^2 was used as the reference.

Statistical analyses were performed using SAS-Enterprise Guide (SAS-EG 7.1) in SAS software, version 9.4 (SAS Institute, Cary, NC, United States). P < 0.05 was considered to represent statistical significance. The statistical methods of this study were reviewed by Dr. Hiroto Narimatsu from the Kanagawa Cancer Center Research Institute, Yokohama, Japan.

RESULTS

The clinical characteristics of the participants, grouped according to the absence or presence of FBH, are shown in Table 1. FBH was experienced by 1842 participants (0.26% of the total). Women were significantly over-represented in the FBH group and the prevalence of FBH was significantly higher in women (0.35%, n = 1,088) than in men (0.18%, n = 754) (χ^2 test, $P < 0.001$).

BMI, systolic blood pressure, serum triglyceride, and HbA1c were significantly lower in the FBH group than in the non-FBH group ($P < 0.001$). No difference in st-age was found, but the plasma high-density lipoprotein-cholesterol concentration was significantly higher in the FBH group ($P < 0.001$). The prevalence of current smoking and breakfast skipping was significantly higher in the FBH group, whereas that of pharmacotherapy for hypertension, late-night dining, and high alcohol consumption was lower in the FBH group.

Figure 2 shows the results of logistic regression analysis, with adjustment for all the listed potential confounding factors (covariates) in the 695613 participants. Low BMI (≤ 20.9 kg/m^2) was significantly associated with FBH, compared with the reference BMI category of 21-22.9 kg/m^2. The OR of BMI ≤ 16.9 kg/m^2 for FBH was almost four, compared with the reference BMI. By contrast, BMI ≥ 23.0 kg/m^2 was inversely associated with FBH. Female sex, current smoking, a history of cardiovascular disease, pharmacotherapy for dyslipidemia, habitual exercise, and breakfast skipping were also significantly associated with FBH. Being in one's 50s and mild-to-moderate alcohol consumption (23-68 g ethanol/day), but not heavy consumption, were inversely associated with FBH, compared with being in one's 40s and a small amount of alcohol consumption (< 23 g ethanol/day), respectively.

However, the relationships of FBH with several of these factors were changed when data from men and women were analyzed separately. Among the men (Figure 3), those in their 60s and 70s were more likely to have FBH. Pharmacotherapy for either hypertension or dyslipidemia was associated with FBH. Additionally, the inverse association of FBH with mild-to-moderate alcohol consumption was also present in men alone. By contrast, among women (Figure 4), those aged ≥ 50 were less likely to have FBH. A history of cardiovascular disease was significantly associated with FBH. However, mild-to-moderate alcohol consumption was not significantly associated with FBH in women. Finally, the associations of FBH with habitual exercise and breakfast skipping almost disappeared when data from men and women were analyzed separately (Figures 3 and 4), although both associations were marginally significant in women.

DISCUSSION

Hypoglycemia frequently occurs in diabetic patients undergoing pharmacotherapy and is difficult to predict[1-4]. In the present study, we have demonstrated that FBH also occurs in non-diabetic people, although the prevalence was very low (less than 1.0% of the total) in the general population studied, in contrast to that in diabetic patients[12,13]. This prevalence of FBH is not dissimilar to that of hypoglycemia (0.2%-0.5%) in non-diabetic people[3,5]. Among the potential confounding factors considered, low body mass and smoking were robustly associated with FBH in both men and women, and across the entire group, women appeared more likely to experience FBH than men. However, when data from each sex were analyzed separately, older women were found to be less likely to have FBH, whereas older men were more likely. The relationships of FBH with other factors (pharmacotherapy, a history of cardiovascular disease, and alcohol consumption) also differed between men and women. Habitual exercise and breakfast skipping were associated with FBH across the entire group, although these associations disappeared when men and women were analyzed separately, probably due to the lower statistical power.

Consistent with our findings, many previous studies have shown associations between low body mass and hypoglycemia in patients with diabetes[14-16]. Additionally, severe hypoglycemia has been shown to be prevalent in people with diabetes who are habitual smokers[17,18]. Therefore, common mechanisms may mediate the relationships between hypoglycemia, low body mass, and habitual smoking, regardless of the type of diabetes present.

FBH can occur as a reactive hypoglycemia in the non-diabetic population[19,20]. For example, reactive hypoglycemia caused by the dumping syndrome, with an incretin-driven insulin hypersecretory response occurs within a few hours of eating a meal in

Table 1 Characteristics of the participants, categorized according to the presence or absence of fasting biochemical hypoglycemia

	No FBH	FBH
n (% of total)	693771 (99.7)	1842 (0.26)
st-Age (yr)	54.7 ± 10.1	54.7 ± 10.7
40s, *n* (%)	269439 (99.7)	758 (0.28)
50s, *n* (%)	185374 (99.8)	418 (0.22)
60s + 70s, *n* (%)	238958 (99.7)	666 (0.28)
Women, *n* (%)	304243 (43.9)	1088 (59.1)
BMI (kg/m^2)	22.9 ± 3.3	21.2 ± 3.4[c]
Systolic blood pressure (mmHg)	122 ± 16.8	118 ± 17.8[c]
High-density lipoprotein-cholesterol (mg/dL)	64.2 ± 16.8	70.6 ± 19.0[c]
Triglyceride, IQR (mg/dL)	90 (64-131)	71 (49-108)[c]
Fasting plasma glucose (mg/dL)	94.3 ± 10.0	64.7 ± 5.6
HbA1c (%)	5.5 ± 0.4	5.4 ± 0.6[c]
Pharmacotherapy for hypertension, *n* (%)	120461 (17.4)	282 (15.3)[a]
Pharmacotherapy for dyslipidemia, *n* (%)	77208 (11.1)	213 (11.6)
Cardiovascular disease, *n* (%)	21410 (3.1)	71 (3.9)
Current smoking, *n* (%)	146253 (21.1)	492 (26.7)[c]
Habitual exercise, *n* (%)[1]	217322 (31.3)	597 (32.4)
Breakfast skipping, *n* (%)[3]	102669 (14.8)	309 (16.8)[a]
Late night dinner, *n* (%)[4], N = 689639	205062 (29.8)	482 (26.3)[b]
Alcohol consumption per day (g ethanol)		
< 23 g	379830 (54.8)	1167 (63.4)
23-45 g	193102 (27.8)	421 (22.9)
46-68 g[2]	88021 (12.7)	173 (9.4)
≥ 69 g[2]	32818 (4.7)	81 (4.4)[c]

Differences in continuous and categorical variables between the two groups were evaluated using Student's *t*-test or the χ^2 test, as appropriate. The trends with increasing alcohol consumption were evaluated using the Cochran-Armitage test.

[a]$P < 0.05$.

[b]$P < 0.01$.

[c]$P < 0.001$.

[1]Defined as habitual exercise to a light sweat for over 30 min per session twice weekly.

[2]Defined as a daily alcohol consumption of ≥ 46 g ethanol.

[3]Defined as skipping breakfast at least three times per week.

[4]Defined as eating dinner within the 2 h preceding bedtime at least three times per week. st-Age: Substituted age; BMI: Body mass index; IQR: Interquartile ratio; FBH: Fasting biochemical hyperglycemia.

people who have undergone gastric or esophageal surgery[21,22]. However, because we excluded individuals from the present study who ate within the 10 h preceding blood sampling, which was established using individual questionnaires, the FBH of the individuals in the present study is unlikely to represent this type of reactive hypoglycemia, which often accompanies hyperinsulinemia[19,20]. However, hypoglycemia in the early morning may be caused by hepatic dysfunction and low gluconeogenesis, both in non-diabetic and diabetic individuals[23,24].

Many conditions and diseases are considered to contribute to the incidence of hypoglycemia in non-diabetic people[24,25]. For example, adrenal insufficiency, use of pain-relieving medication, malnutrition or low food intake, infection, low hepatic gluconeogenic capacity, and low glycogen storage have all been implicated.

The reasons why women and older men were more likely to have FBH are unknown. A plausible explanation for the former is that the insulin sensitivity of women is higher, whereas their hepatic gluconeogenesis is lower because of higher

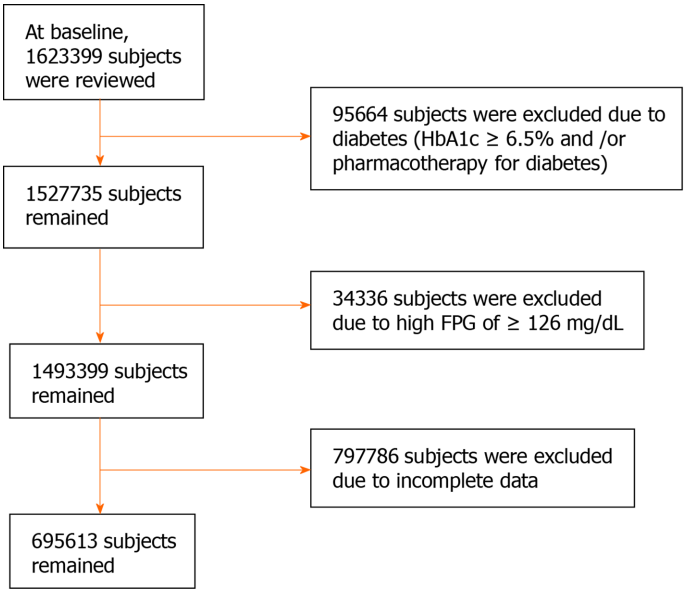

Figure 1 Exclusion criteria and participant disposition. The exclusion criteria and participant flow chart are shown. FPG: Fasting plasma glucose.

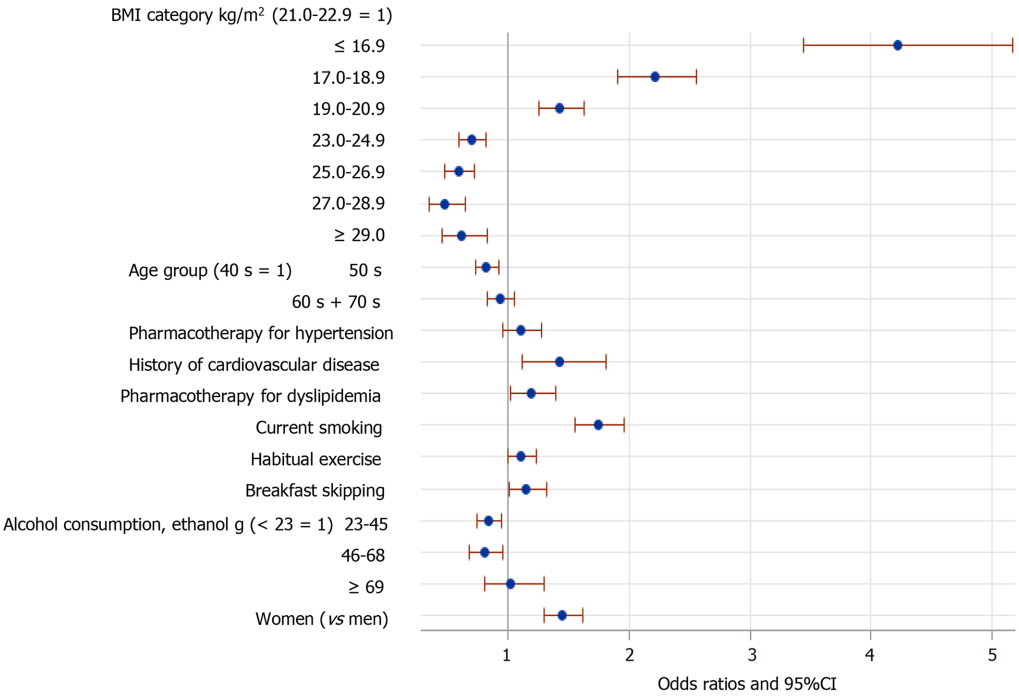

Figure 2 Odds ratios and 95% confidence interval for the relationships between various parameters and fasting biochemical hypoglycemia across the entire group. BMI: Body mass index; CI: Confidence interval.

circulating 17β-estradiol concentrations in women than men[26,27]. Furthermore, older men may have a lower gluconeogenic capacity than younger men[28,29], which would result in an inadequate supply of blood glucose in the early morning before breakfast. The insulin sensitivity of individuals who habitually exercise may also be higher, possibly contributing to lower blood glucose concentrations. However, it is unknown whether such factors specifically influence the incidence of FBH.

Acute alcohol consumption increases the risk of hypoglycemia through the suppression of gluconeogenesis in the liver, which is more likely to occur in the fasting state in both diabetic and non-diabetic people[30-32]. However, the long-term effect of alcohol consumption is poorly understood[32].

In the present study, heavy alcohol consumption (≥ 69 g ethanol/day) was not associated with FBH, whereas mild-to-moderate consumption was inversely associated with FBH, particularly in men. A plausible explanation for this is that

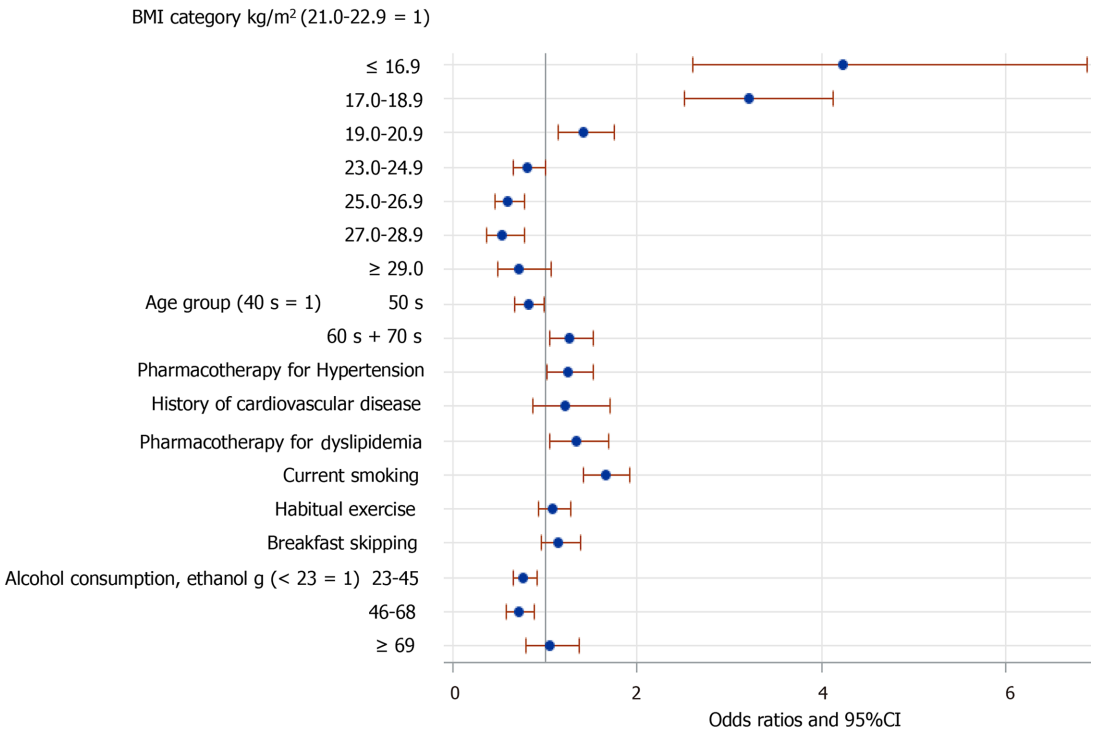

Figure 3 Odds ratios and 95% confidence interval for the relationships between various parameters and fasting biochemical hypoglycemia in men. BMI: Body mass index; CI: Confidence interval.

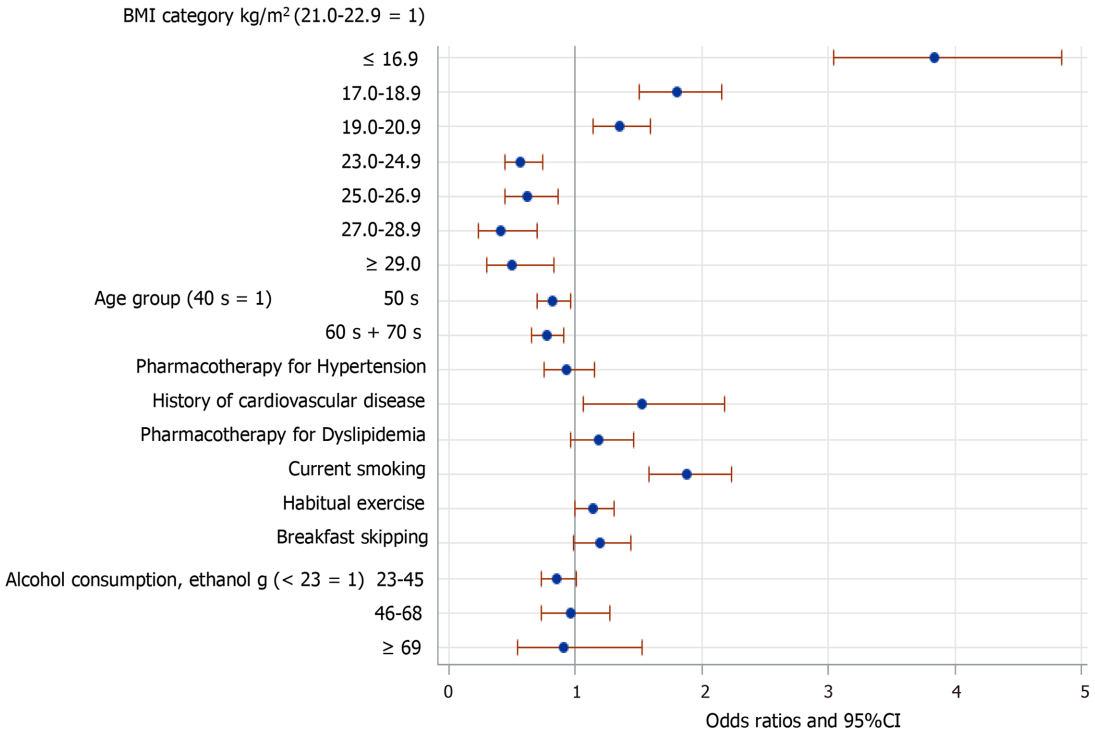

Figure 4 Odds ratios and 95% confidence interval for the relationships between various parameters and fasting biochemical hypoglycemia in women. BMI: Body mass index; CI: Confidence interval.

individuals who drink mild-to-moderate amounts of alcohol may concurrently consume an inadequate amount of food, predisposing toward subsequent hypoglycemia. In heavy drinkers, carbohydrate intake and glycogen storage may be inadequate, which could result in hypoglycemia several hours later, in addition to their lower renal and hepatic gluconeogenesis[31,32].

The reasons why breakfast skipping and pharmacotherapy for hypertension and dyslipidemia tended to be associated with FBH are unknown. A plausible reason for the former is that the sensitivity to hypoglycemia may be low, such that it does not elicit hunger and consequently removes the perceived need for the consumption of breakfast. In contrast, the lower prevalence of late-night dining in the FBH group (Table 1) may be less prevalent because the consumption of carbohydrates before sleep prolongs the peak blood glucose concentration until early morning.

Although we have shown that FBH occurs even in non-diabetic people, its clinical relevance in this group remains to be more fully determined. Although higher mortality in hospital[6,33], traffic accidents due to cognitive dysfunction[34], and fatal arrhythmias such as QT prolongation[35] are more prevalent in non-diabetic individuals who experience hypoglycemia, it is unknown whether these problems are also more common in non-diabetic people who experience FBH, and this deserves further study.

The main strength of the present study was that the FPG concentration was measured using a standard laboratory method, rather than using portable glucose meters, which can sometimes be inaccurate[36,37]. Furthermore, we were unable to conduct the present analysis until we had collected data from hundreds of thousands of people because of the very low prevalence of FBH. To the best of our knowledge, this study is the first to determine the prevalence of FBH and identify the associated factors in a non-diabetic population.

The present study also had several limitations. First, the assessment of hypoglycemia was conducted just once, using plasma samples obtained after overnight fasting. Therefore, it is unknown whether the prevalence of calculated FBH represents a transient or persistent phenomenon. Furthermore, the prevalence of hypoglycemia, including reactive hypoglycemia, during the daytime was not assessed during the present study. Second, the diet of the participants was not assessed in the present study, which prevented investigation of the relationships between the amounts of carbohydrates and energy consumed with FBH. Finally, we cannot draw conclusions with respect to cause and effect using the data collected because the study was cross-sectional. To identify causal relationships, a prospective, large-scale study of non-diabetic people should be conducted in the future.

CONCLUSION

We have shown that FBH occurs even in non-diabetic people, albeit very infrequently. FBH appears to be robustly associated with low body mass and smoking. Women and men in their 60s and 70s were more likely to experience FBH, and the relationships between FBH and other factors also differed between men and women.

ARTICLE HIGHLIGHTS

Research background

The clinical relevance and the prevalence of fasting biochemical hypoglycemia (FBH) are poorly understood in a general population without diabetes.

Research motivation

FBH can be influenced by many factors, including age, sex, body mass, smoking, alcohol drinking, exercise levels, medications, and eating behaviors.

Research objectives

We determined the prevalence of FBH and investigated its association with potential risk factors in a population who did not have diabetes.

Research methods

In a cross-sectional study of 695613 people aged 40-74 years who had undergone a health check-up, clinical parameters and lifestyle-related factors were reviewed. FBH was defined as a fasting plasma glucose < 70 mg/dL (3.9 mmol/L) after overnight fasting, regardless of any symptoms.

Research results

The prevalence of FBH was very low (0.26%) in this study. A body mass index of ≤ 20.9 kg/m^2 and current smoking were significantly associated with FBH, which was not altered by adjustment for age, sex, and pharmacotherapy for hypertension or dyslipidemia. When the data were analyzed according to sex, men in their 60s or 70s appeared more likely to experience FBH compared with their 40s, whereas men in their 50s and women aged ≥ 50 years appeared less likely to experience FBH.

Research conclusions

FBH was observed even in non-diabetic people, albeit at a very low frequency. FBH is robustly associated with low body mass and smoking, and its relationship with lifestyle factors varies according to sex.

Research perspectives

To identify causal relationships between FBH and relevant factors (underweight, smoking, men in their 60s and so on), a prospective, large-scale study of non-diabetic people should be conducted in the future.

REFERENCES

1 **Zammitt NN**, Frier BM. Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. *Diabetes Care* 2005; **28**: 2948-2961 [PMID: 16306561 DOI: 10.2337/diacare.28.12.2948]

2 **Shafiee G**, Mohajeri-Tehrani M, Pajouhi M, Larijani B. The importance of hypoglycemia in diabetic patients. *J Diabetes Metab Disord* 2012; **11**: 17 [PMID: 23497433 DOI: 10.1186/2251-6581-11-17]

3 **Silbert R**, Salcido-Montenegro A, Rodriguez-Gutierrez R, Katabi A, McCoy RG. Hypoglycemia Among Patients with Type 2 Diabetes: Epidemiology, Risk Factors, and Prevention Strategies. *Curr Diab Rep* 2018; **18**: 53 [PMID: 29931579 DOI: 10.1007/s11892-018-1018-0]

4 **Pratiwi C**, Mokoagow MI, Made Kshanti IA, Soewondo P. The risk factors of inpatient hypoglycemia: A systematic review. *Heliyon* 2020; **6**: e03913 [PMID: 32420485 DOI: 10.1016/j.heliyon.2020.e03913]

5 **Nirantharakumar K**, Marshall T, Hodson J, Narendran P, Deeks J, Coleman JJ, Ferner RE. Hypoglycemia in non-diabetic in-patients: clinical or criminal? *PLoS One* 2012; **7**: e40384 [PMID: 22768352 DOI: 10.1371/journal.pone.0040384]

6 **Sako A**, Yasunaga H, Matsui H, Fushimi K, Hamasaki H, Katsuyama H, Tsujimoto T, Goto A, Yanai H. Hospitalization with hypoglycemia in patients without diabetes mellitus: A retrospective study using a national inpatient database in Japan, 2008-2012. *Medicine (Baltimore)* 2017; **96**: e7271 [PMID: 28640135 DOI: 10.1097/MD.0000000000007271]

7 **Mongraw-Chaffin M**, Beavers DP, McClain DA. Hypoglycemic symptoms in the absence of diabetes: Pilot evidence of clinical hypoglycemia in young women. *J Clin Transl Endocrinol* 2019; **18**: 100202 [PMID: 31428564 DOI: 10.1016/j.jcte.2019.100202]

8 **Nakajima K**, Iwane T, Higuchi R, Shibata M, Takada K, Uda J, Anan M, Sugiyama M, Nakamura T. Kanagawa Investigation of the Total Check-up Data from the National database (KITCHEN): protocol for data-driven population-based repeated cross-sectional and 6-year cohort studies. *BMJ Open* 2019; **9**: e023323 [PMID: 30796118 DOI: 10.1136/bmjopen-2018-023323]

9 **Conceptual Diagram**. Provision of Medical-Related Data to a Third Party. [cited 7 January 2021]. Available from: https://www.mhlw.go.jp/content/12401000/000402528.pdf

10 **Rozance PJ**, Hay WW Jr. Describing hypoglycemia--definition or operational threshold? *Early Hum Dev* 2010; **86**: 275-280 [PMID: 20554129 DOI: 10.1016/j.earlhumdev.2010.05.002]

11 **Nakajima K**, Higuchi R, Mizusawa K. Unexpectedly High Prevalence of Breakfast Skipping in Low Body-Weight Middle-Aged Men: Results of the Kanagawa Investigation of Total Checkup Data from the National Data Base-7 (KITCHEN-7). *Nutrients* 2020; **13** [PMID: 33396798 DOI: 10.3390/nu13010102]

12 **Miller CD**, Phillips LS, Ziemer DC, Gallina DL, Cook CB, El-Kebbi IM. Hypoglycemia in patients with type 2 diabetes mellitus. *Arch Intern Med* 2001; **161**: 1653-1659 [PMID: 11434798 DOI: 10.1001/archinte.161.13.1653]

13 **Saito T**, Ohmura H, Nojiri S, Daida H. Impact of sitagliptin combination therapy and hypoglycemia in Japanese patients with type 2 diabetes: a multi-center retrospective observational cohort study. *J Pharm Health Care Sci* 2020; **6**: 13 [PMID: 32518665 DOI: 10.1186/s40780-020-00169-5]

14 **Sasaki M**, Mogi T, Wada Y, Hirosawa I, Koizumi A. An endemic condition of biochemical hypoglycemia among male volunteers. *Ind Health* 1996; **34**: 323-333 [PMID: 8908843 DOI: 10.2486/indhealth.34.323]

15 **Cheng PC**, Hsu SR, Tu ST, Cheng YC, Liu YH. Body mass index influences the plasma glucose concentration during iatrogenic hypoglycemia in people with type 2 diabetes mellitus: a cross-sectional study. *PeerJ* 2018; **6**: e4348 [PMID: 29473001 DOI: 10.7717/peerj.4348]

16 **Han K**, Yun JS, Park YM, Ahn YB, Cho JH, Cha SA, Ko SH. Development and validation of a risk

prediction model for severe hypoglycemia in adult patients with type 2 diabetes: a nationwide population-based cohort study. *Clin Epidemiol* 2018; **10**: 1545-1559 [PMID: 30425585 DOI: 10.2147/CLEP.S169835]

17 **Hirai FE**, Moss SE, Klein BE, Klein R. Severe hypoglycemia and smoking in a long-term type 1 diabetic population: Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Diabetes Care* 2007; **30**: 1437-1441 [PMID: 17372156 DOI: 10.2337/dc06-2264]

18 **Szwarcbard N**, Villani M, Earnest A, Flack J, Andrikopoulos S, Wischer N, Soldatos G, Gasevic D, Zoungas S. The association of smoking status with glycemic control, metabolic profile and diabetic complications- Results of the Australian National Diabetes Audit (ANDA). *J Diabetes Complications* 2020; **34**: 107626 [PMID: 32527672 DOI: 10.1016/j.jdiacomp.2020.107626]

19 **Eckert-Norton M**, Kirk S. Non-diabetic hypoglycemia. *J Clin Endocrinol Metab* 2013; **98**: 39A-40A [PMID: 24098022 DOI: 10.1210/jc.2013-v98i10.39A]

20 **Altuntaş Y**. Postprandial Reactive Hypoglycemia. *Sisli Etfal Hastan Tip Bul* 2019; **53**: 215-220 [PMID: 32377086 DOI: 10.14744/SEMB.2019.59455]

21 **Tack J**, Arts J, Caenepeel P, De Wulf D, Bisschops R. Pathophysiology, diagnosis and management of postoperative dumping syndrome. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 583-590 [PMID: 19724252 DOI: 10.1038/nrgastro.2009.148]

22 **van Beek AP**, Emous M, Laville M, Tack J. Dumping syndrome after esophageal, gastric or bariatric surgery: pathophysiology, diagnosis, and management. *Obes Rev* 2017; **18**: 68-85 [PMID: 27749997 DOI: 10.1111/obr.12467]

23 **Frizzell RT**, Campbell PJ, Cherrington AD. Gluconeogenesis and hypoglycemia. *Diabetes Metab Rev* 1988; **4**: 51-70 [PMID: 3278874 DOI: 10.1002/dmr.5610040107]

24 **Anno T**, Kaneto H, Shigemoto R, Kawasaki F, Kawai Y, Urata N, Kawamoto H, Kaku K, Okimoto N. Hypoinsulinemic hypoglycemia triggered by liver injury in elderly subjects with low body weight: case reports. *Endocrinol Diabetes Metab Case Rep* 2018; **2018** [PMID: 29497536 DOI: 10.1530/EDM-17-0155]

25 **Gosmanov AR**, Gosmanova EO, Kovesdy CP. Evaluation and management of diabetic and non-diabetic hypoglycemia in end-stage renal disease. *Nephrol Dial Transplant* 2016; **31**: 8-15 [PMID: 26152404 DOI: 10.1093/ndt/gfv258]

26 **Brussaard HE**, Gevers Leuven JA, Frölich M, Kluft C, Krans HM. Short-term oestrogen replacement therapy improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM. *Diabetologia* 1997; **40**: 843-849 [PMID: 9243107 DOI: 10.1007/s001250050758]

27 **Yan H**, Yang W, Zhou F, Li X, Pan Q, Shen Z, Han G, Newell-Fugate A, Tian Y, Majeti R, Liu W, Xu Y, Wu C, Allred K, Allred C, Sun Y, Guo S. Estrogen Improves Insulin Sensitivity and Suppresses Gluconeogenesis *via* the Transcription Factor Foxo1. *Diabetes* 2019; **68**: 291-304 [PMID: 30487265 DOI: 10.2337/db18-0638]

28 **Podolin DA**, Gleeson TT, Mazzeo RS. Role of norepinephrine in hepatic gluconeogenesis: evidence of aging and training effects. *Am J Physiol* 1994; **267**: E680-E686 [PMID: 7977718 DOI: 10.1152/ajpendo.1994.267.5.E680]

29 **Kim K**, Cho SC, Cova A, Jang IS, Park SC. Alterations of epinephrine-induced gluconeogenesis in aging. *Exp Mol Med* 2009; **41**: 334-340 [PMID: 19307753 DOI: 10.3858/emm.2009.41.5.037]

30 **Kubota M**, Virkamäki A, Yki-Järvinen H. Ethanol stimulates glycogenolysis in livers from fed rats. *Proc Soc Exp Biol Med* 1992; **201**: 114-118 [PMID: 1528905 DOI: 10.3181/00379727-201-43488]

31 **van de Wiel A**. Diabetes mellitus and alcohol. *Diabetes Metab Res Rev* 2004; **20**: 263-267 [PMID: 15250029 DOI: 10.1002/dmrr.492]

32 **Sumida KD**, Hill JM, Matveyenko AV. Sex differences in hepatic gluconeogenic capacity after chronic alcohol consumption. *Clin Med Res* 2007; **5**: 193-202 [PMID: 18056029 DOI: 10.3121/cmr.2007.729]

33 **Mannucci E**, Monami M, Mannucci M, Chiasserini V, Nicoletti P, Gabbani L, Giglioli L, Masotti G, Marchionni N. Incidence and prognostic significance of hypoglycemia in hospitalized non-diabetic elderly patients. *Aging Clin Exp Res* 2006; **18**: 446-451 [PMID: 17167310 DOI: 10.1007/BF03324842]

34 **Noma Y**, Komatsu M, Miya K, Shima K. Cognitive dysfunction during hypoglycemia in an elderly subject without diabetes. *Diabetol Int* 2020; **11**: 150-154 [PMID: 32206485 DOI: 10.1007/s13340-019-00419-4]

35 **Tsujimoto T**, Yamamoto-Honda R, Kajio H, Kishimoto M, Noto H, Hachiya R, Kimura A, Kakei M, Noda M. High risk of abnormal QT prolongation in the early morning in diabetic and non-diabetic patients with severe hypoglycemia. *Ann Med* 2015; **47**: 238-244 [PMID: 25861830 DOI: 10.3109/07853890.2015.1017528]

36 **Klonoff DC**, Parkes JL, Kovatchev BP, Kerr D, Bevier WC, Brazg RL, Christiansen M, Bailey TS, Nichols JH, Kohn MA. Investigation of the Accuracy of 18 Marketed Blood Glucose Monitors. *Diabetes Care* 2018; **41**: 1681-1688 [PMID: 29898901 DOI: 10.2337/dc17-1960]

37 **King F**, Ahn D, Hsiao V, Porco T, Klonoff DC. A Review of Blood Glucose Monitor Accuracy. *Diabetes Technol Ther* 2018; **20**: 843-856 [PMID: 30376362 DOI: 10.1089/dia.2018.0232]

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