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REVIEW

Nonalcoholic fatty liver disease and diabetes

Maria Irene Bellini, Irene Urciuoli, Giovanni Del Gaudio, Giorgia Polti, Giovanni Iannetti, Elena Gangitano, Eleonora Lori, Carla Lubrano, Vito Cantisani, Salvatore Sorrenti, Vito D'Andrea

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease in the world and represents a clinical-histopathologic entity where the steatosis component may vary in degree and may or may not have fibrotic progression. The key concept of NAFLD pathogenesis is excessive triglyceride hepatic accumulation because of an imbalance between free fatty acid influx and efflux. Strong epidemiological, biochemical, and therapeutic evidence supports the premise that the primary pathophysiological derangement in most patients with NAFLD is insulin resistance; thus the association between diabetes and NAFLD is widely recognized in the literature. Since NAFLD is the hepatic manifestation of a metabolic disease, it is also associated with a higher cardiovascular risk. Conventional B-mode ultrasound is widely adopted as a first-line imaging modality for hepatic steatosis, although magnetic resonance imaging represents the gold standard noninvasive modality for quantifying the amount of fat in these patients. Treatment of NAFLD patients depends on the disease severity, ranging from a more benign condition of nonalcoholic fatty liver to nonalcoholic steatohepatitis. Abstinence from alcohol, a Mediterranean diet, and modification of risk factors are recommended for patients suffering from NAFLD to avoid major cardiovascular events, as per all diabetic patients. In addition, weight loss induced by bariatric surgery seems to also be effective in improving liver features, together with the benefits for diabetes control or resolution, dyslipidemia, and hypertension. Finally, liver transplantation represents the ultimate treatment for severe nonalcoholic fatty liver disease and is growing rapidly as a main indication in Western countries. This review offers a comprehensive multidisciplinary approach to NAFLD, highlighting its connection with diabetes.

Key Words: Bariatric surgery; Diabetes; Hepatic steatosis; Liver fibrosis; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis

Core Tip: Nonalcoholic fatty liver disease is the most common liver disease worldwide, characterized by fat accumulation in the hepatic parenchyma, with a range of different stages from mild inflammation to severe fibrosis. There is a biunivocal relationship with type 2 diabetes, with important consequences in terms of cardiovascular risk, which seems to also have occurred during the coronavirus disease 2019 pandemic. This review focuses on the pathogenesis, clinical aspects, and treatment, providing guidance for a non-invasive diagnosis and preferred therapy, medical and/or surgical.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease worldwide[1] and represents a clinico-histopathologic entity with features mimicking alcohol-induced liver injury, but occurring, by definition, in patients with little or no history of alcohol consumption. Its prevalence reaches up to 25%-30%[2,3] of the worldwide population, with approximately 2 billion of individuals being affected[4].

NAFLD includes a different variety of findings, ranging from hepatocyte fat accumulation without concomitant inflammation or fibrosis (simple hepatic steatosis), to hepatic steatosis with a necro-inflammatory component (steatohepatitis), which may or may not have associated fibrosis. Nonalcoholic steatohepatitis (NASH) may progress to cirrhosis in up to 20% of patients[5,6], and it is a leading cause of cryptogenic cirrhosis[7].

The cause of NAFLD has not been fully elucidated and is considered multifactorial. A two-hit model of NAFLD development was originally proposed. The first consists of hepatic steatosis, which then sensitizes the liver to a progressive injury and is mediated by "second hits" as inflammatory cytokines, adipokines, and oxidative stress. Together they lead to steatohepatitis and fibrosis[8]. Currently, the two-hit hypothesis has been replaced by the "multiple hit" theory, which recognizes the following components in NAFLD pathophysiology: insulin resistance, obesity, gut microbiota, and environmental and genetic factors[9].

The aim of this review is to report, from a comprehensive multidisciplinary perspective, the pathogenesis, diagnosis, and treatment of NAFLD, highlighting its relationship with diabetes.

PATHOGENESIS

The key concept of NAFLD pathogenesis is excessive triglyceride hepatic accumulation as a result of an imbalance between free fatty acid (FFA) influx and efflux[10]. This can occur from the excessive importation of FFAs from the adipose tissue; diminished hepatic export of FFA, possibly secondary to reduced synthesis or secretion of very low-density lipoprotein; or the impaired beta-oxidation of FFA. The pathogenesis and evolution of NAFLD are depicted in Figure 1.

Strong epidemiological, biochemical, and therapeutic evidence supports the premise that the primary pathophysiological derangement in most patients with NAFLD is insulin resistance. Resistance to the action of insulin results in important changes in lipid metabolism. These include enhanced peripheral lipolysis, increased triglyceride synthesis, and increased hepatic uptake of fatty acids. Each of these may contribute to the accumulation of hepatocellular triglycerides, which in turn results in a preferential shift from carbohydrate to FFA beta-oxidation, an occurrence that has been demonstrated in patients with insulin resistance[11]. The association of liver steatosis and metabolic dysfunction is so strict that a new definition was recently proposed to define this entity, namely "metabolic (dysfunction)-associated fatty liver disease" (MAFLD)[12].

The excessive inflow of triglycerides to the liver leads to inflammation, reactive oxygen species (ROS) formation, hepatocyte impaired function, and lipotoxicity. Hepatocellular cells injury activates apoptotic pathways, ultimately causing cellular death. This results in the progression from noninflammatory

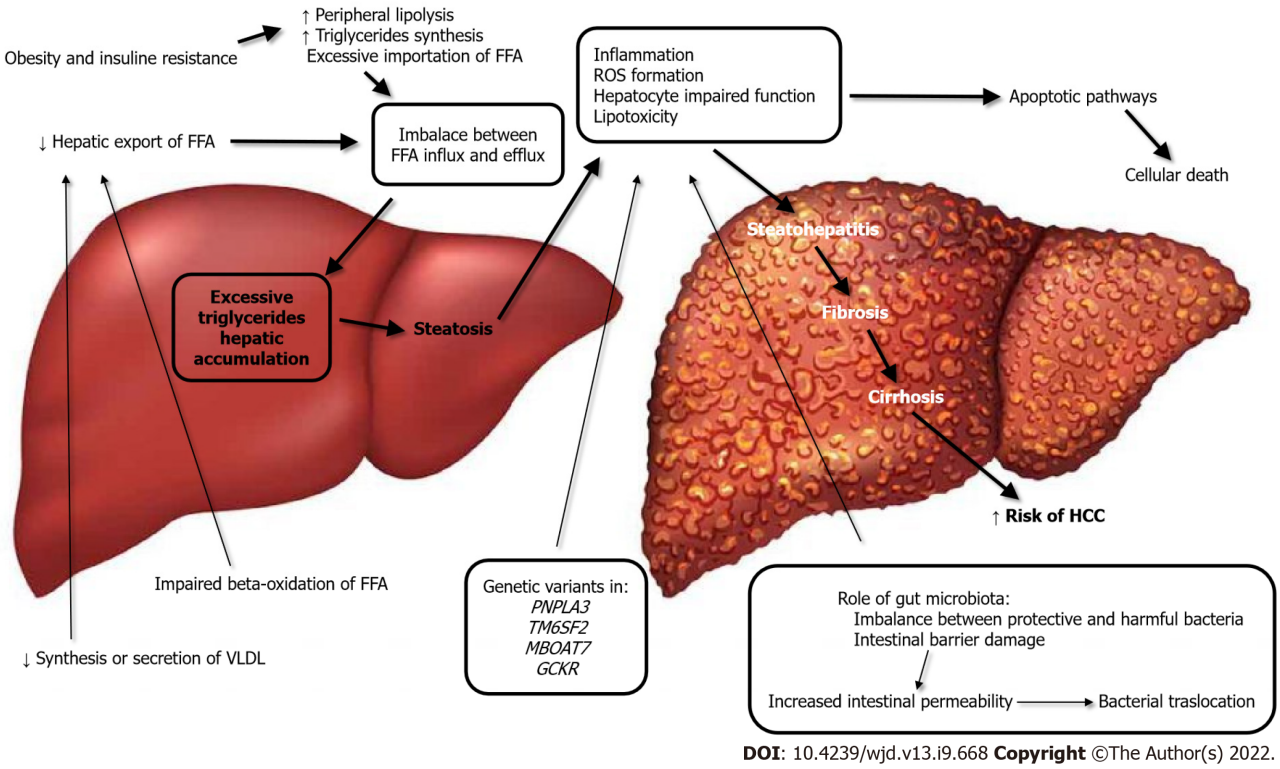

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Figure 1 Pathogenesis and evolution of nonalcoholic fatty liver disease. FFA: Fatty free acids; HCC: Hepatocellular carcinoma; ROS: Reactive oxygen species; VLDL: Very low-density lipoprotein.

isolated steatosis to the development of nonalcoholic steatohepatitis, with a risk of further evolution to fibrosis, cirrhosis and, worst-case scenario, to the development of hepatocellular carcinoma[9,13]. In this regard, the major role of mitochondrial dysfunction in the genesis of NAFLD has emerged in recent years; in fact mitochondria are responsible for the β-oxidation of FFAs and controlling the tricarboxylic acid cycle. Furthermore, mitochondria favor cell adaption to oxidative stress, mitigating the effects of ROS production[14].

Intestinal microbes have also been implicated as a potential source of hepatotoxic oxidative injury, and changes in the microbiome play a role in the lipotoxicity and pathogenesis of NAFLD[15,16].

The specific composition of gut microbiota may play a role in both the inflammatory and fibrosis responses in patients with NAFLD. The imbalance between protective and harmful bacteria, such as altered *Firmicutes/Bacteroidetes* ratio, relative abundance of alcohol-producing bacteria, growth of harmful genera, and lack of protective genera, together predispose[17] to damage of the intestinal barrier. The consequent epithelial disruption leads to an altered immune reaction and activation of inflammatory pathways, as a response to the bacterial products, namely short-chain fatty acids, trimethylamine N-oxide, and secondary bile acids[18]. Damage of the intestinal membrane finally results in impaired transport across the mucosa, increasing the filtration of bacterial lipopolysaccharides and thus further contributing to NAFLD development[17,19].

In terms of genetic risk factors, there is also a role in the development of NAFLD. Studies on twins have demonstrated a strong hereditary correlation, estimated to be approximately 50%, to both hepatic fat content and hepatic fibrosis[4]. It is recognized that at least four genetic variants in four different genes (PNPLA3, TM6SF2, MBOAT7, and GCKR) are responsible for the encoding of hepatic lipid metabolism regulatory proteins and are therefore involved in the development and progression of NAFLD[12,20].

DIABETES AND NAFLD: A WELL-ESTABLISHED RELATIONSHIP

Among type 2 diabetes (T2D) patients, the prevalence of NAFLD is more than double compared to the general population, and is estimated to be over 55%. The global prevalence of NASH in T2D patients is 37%[1]. The prevalence of NAFLD in T1D is reportedly between 10% and 20%[21,22].

The association between T2D and NAFLD is widely recognized in the literature[23-26]. T2D is itself a risk factor for the development of NAFLD, and seems to accelerate the progression of liver disease[1,27]. On the other hand, NAFLD is a risk factor for the development of T2D and its complications[22,23,27-29]. In fact, NAFLD gives a two-fold increased risk of incident diabetes over a course of about 5 years

[23,30], and the risk of patients affected by liver steatosis to develop diabetes increases in parallel to the extent of steatosis severity[30], becoming even higher when the fibrosis is advanced[23,30].

A study on 2020 participants, with a 10-year follow-up, observed that the fatty liver index (FLI), an indirect assessment used to quantify the amount of hepatic fat with a mathematical formula, predicts incident risk of developing T2D and glycemic alterations preceding diabetes. Individuals with a high FLI had an increased risk of developing diabetes, and among these high FLI patients, overweight and obese people had a risk that increased by more than 10- and 15-fold compared to similar body mass index-matched people but lower FLI[31]. Similarly, another study on 28991 pre-diabetic patients with a 3-year follow-up found that high FLI is a risk factor for developing diabetes, even in nonobese patients [32]. Of note, NAFLD predicts the development of metabolic syndrome over a period of less than 5 years[33], and metabolic syndrome is considered a risk factor for T2D.

NAFLD is associated with the development of macrovascular and microvascular complications in T2D patients, including chronic kidney disease (CKD)[29], retinopathy and autonomic neuropathy, although the results across studies are not completely concordant[34,35]. Liver fibrosis is also independently associated with macrovascular and microvascular complications in diabetic patients[36], and although T2D is a well-known risk factor to CKD, NAFLD predicts deterioration of renal function even in healthy subjects.

As per dietary advice, adherence to a Mediterranean diet is inversely associated with NAFLD and prevents the development of T2D and cardiovascular disease (CVD) in patients with NAFLD over a 10-year span[37], whereas the low adherence to these food habits is associated with diabetes and CVD onset in NAFLD patients[38]. Virtually, most studies assessing liver fat content have reported positive results after very low-calorie diets and ketogenic diets. While it is acknowledged that weight loss is associated with amelioration of NAFLD, less is known about the effect of macronutrient distribution on such outcomes. Carbohydrate restriction, with its well-established role in modulating insulin levels, and the newly proposed pathway involving the microbiome shift with increased folate production, likely plays a primary role in the reported effectiveness of ketogenic diets towards NAFLD[39].

Figure 2 summarizes the pathophysiological link between NAFLD and T2D.

DIABETES, NAFLD, AND CARDIOVASCULAR RISK

CVD is among the leading causes of death worldwide[40], and the prevention of cardiovascular events is crucial from a global health perspective.

Atherosclerotic CVD is the major cause of morbidity and mortality in diabetic patients[41]. CVD comorbidities often present in diabetic patients as hypertension and dyslipidemia, are additive risk factors for cardiovascular events. T2D is a recognized cardiovascular risk factor as well, and NAFLD contributes independently to CVD[42].

Since NAFLD is the hepatic manifestation of a metabolic disease, it is also associated with a higher cardiovascular risk[43]. A recent meta-analysis assessed the long-term higher risk of fatal and nonfatal CVD events, observing an increase across steatosis stages, reaching the maximum when fibrosis was present[44]. NAFLD is also significantly associated with hypertension[45] and heart failure[46], thus significantly increasing the overall mortality risk[46]. In a retrospective study comparing more than 900 subjects affected either by NAFLD or AFLD or with normal liver appearance on computed tomography, fatty liver independently from the cause of the steatosis was associated with a higher cardiovascular risk [47]. Since NAFLD is a dynamic entity, it is, by definition, subject to variation over time. In the same study, Lee *et al*[47] evaluated 3 million subjects for NAFLD with FLI for a minimum of four times, between 2009 and 2013, concluding that higher persistent FLI led to a higher mortality rate for all causes, myocardial infarction, and stroke. These results were confirmed after correcting for many possible confounders such as age, sex, smoking, alcohol consumption, income, dyslipidemia, body mass index, diabetes, hypertension, and physical activity[47].

As already discussed, diabetes and NAFLD are often associated; thus they may act synergistically to maximally increase cardiovascular risk[48]; the higher incidence of CVD in diabetic patients with steatosis compared to diabetic patients without steatosis[48] seems to confirm this detrimental association.

A study on > 130000 T2D patients with a hospital record of NAFLD or AFLD, and no record of any other liver disease, showed an increased risk for recurrent CVD, cancer, and mortality for all causes[49]. Patients with a history of hospital admission and fatty liver were younger than those without liver disease[50]. Of note, similar to what happens in healthy subjects and T2D patients, even in T1D patients, NAFLD increases the cardiovascular risk[51].

Figure 3 illustrates the association of T2D and NAFLD with multiple morbid conditions; thus the coexistence and interaction of the two, further exacerbates the prognosis of each.

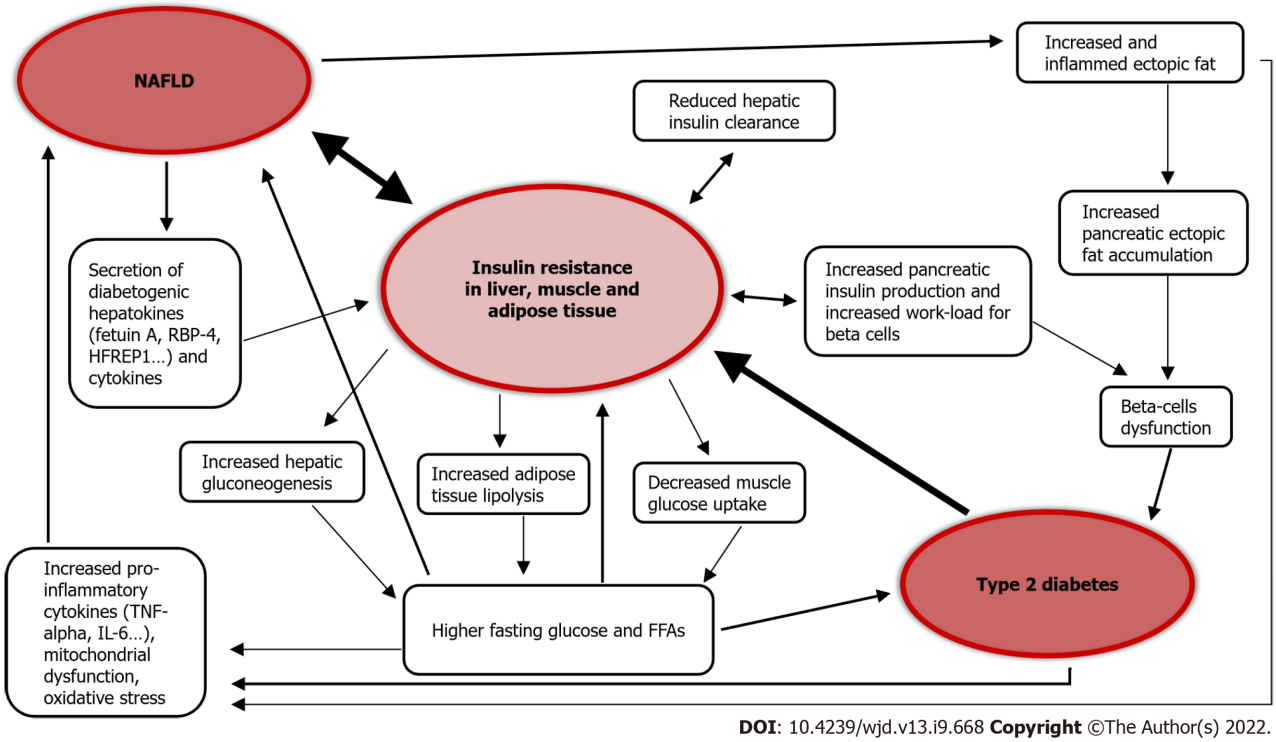

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Figure 2 The link between nonalcoholic fatty liver disease and diabetes pathogenesis. Nonalcoholic fatty liver disease increases the risk of developing type 2 diabetes mainly through worsening insulin resistance and increasing gluconeogenesis. By contrast, type 2 diabetes increases the risk of developing liver steatosis and fibrosis through insulin resistance, oxidative stress, and inflammatory cytokines.

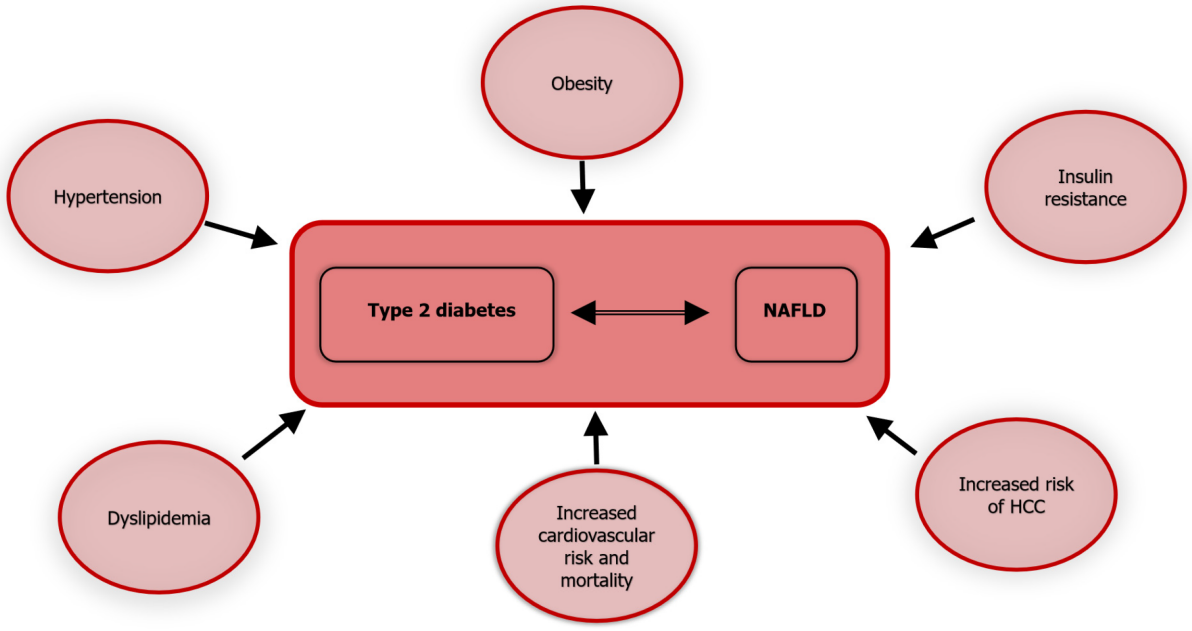

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Figure 3 Type 2 diabetes and nonalcoholic fatty liver disease are both associated with multiple metabolic and cardiovascular morbidities. Furthermore, the presence of one increases the risk to develop the other and thus exacerbating the overall prognosis. HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease.

DIABETES, NAFLD, AND CORONAVIRUS DISEASE 2019

From the very beginning of the severe acute respiratory syndrome coronavirus 2 pandemic, diabetes has shown an association to this virus infection. In fact, a study on 5700 patients admitted to 12 hospitals in the New York City area demonstrated that the most common comorbidities in admitted coronavirus

disease 2019 (COVID-19) patients were hypertension (56.6%), obesity (41.7%), and diabetes (33.8%)[52]. Diabetes prevalence in COVID-19 patients is high, varying from 15%, in a pool of more than 23000 patients[53], up to almost 40% in another study on 200 hospitalized patients[54].

Diabetic patients have a higher risk of contracting COVID-19[55], a higher risk of hospitalization[54] and mortality[56].

NAFLD is also associated to COVID-19[57], to its severity progression, risk of intubation, dialysis and use of vasopressors[58], although in contrast, some authors[59-61] did not observe a higher risk of severe COVID-19 and intensive care unit access for NAFLD patients.

A longer viral shedding time[62] and a higher mortality for COVID-19 in NASH patients with advanced fibrosis[63] have also been reported.

NAFLD DIAGNOSIS

NAFLD diagnosis is based on three criteria: (1) Absence of significant alcohol intake; (2) presence of hepatic steatosis; and (3) exclusion of other causes of liver disease.

Some clinical biomarkers are used to screen for or diagnose NAFLD, used in complex algorithms for risk stratification. They aim to combine various conditions, such as arterial hypertension with laboratory exams, like transaminases, to predict outcomes of the liver disease, but as single markers, they only provide poor sensitivity and specificity. Yet, their overall performance is limited, with further studies needed to transfer the initial thought cut-off values into the real clinical scenario[64].

It can therefore be asserted that due to the lack of available noninvasive methods to confirm the diagnosis of NAFLD, liver biopsy remains the gold standard to classify steatosis, and NASH. However, biopsy has limitations[65]; namely it is invasive, subject to sampling variability and observer-dependence, and most importantly, carries risks. Therefore, it is not offered to routinely assess the amount of fatty liver in NAFLD patients who may have simple steatosis, as reported in the majority of cases[6].

As previously mentioned, since NAFLD is a dynamic entity[47], varying through lifetime, imaging methods remain the most widely utilized tools to assess NAFLD patients and quantify the relative hepatic steatosis.

NAFLD IMAGING

To date, various imaging methods have been utilized: ultrasonography, CT, magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS). More recently, other diagnostic tools measuring liver stiffness have entered clinical practice, in view of their practical utility, as reported in Table 1.

Ultrasound

Conventional B-mode ultrasound (US) is the most widely used imaging modality for the noninvasive evaluation of hepatic steatosis, as first-line diagnostic imaging procedure, according to clinical practice guidelines[66]. Fatty liver infiltration is characterized by hyperechogenicity of the parenchyma and increasing attenuation of US waves in deeper parts, specifically where there is increasing steatosis[67]. However, US evaluation of fatty livers is based on the operator's experience; in comparison to histology as reference standard, the overall sensitivity and specificity of B-mode US are, respectively, 84.8% and 93.6%, with 0.93 accuracy[68].

US elastography quantitatively evaluates liver stiffness. Two broad categories of imaging-based sonoelastography are currently in clinical use: strain elastography, which is influenced by the operator or physiologic forces that produce tissue deformation; and shear wave elastography (SWE), which instead results from the acoustic radiation force of the tissue displacement[69,70].

Fibroscan uses transient US elastography (TE) to measure hepatic elasticity by quantifying the shear wave velocity with ultrasonic echo pulses from low-frequency vibrations that are transmitted into the liver[71,72]. Since patients with > 66% steatosis at liver biopsy have a false-positive higher rate, *via* the Fibroscan XL probe it is also possible to investigate obese patients, given that during TE the transmission of a mechanical wave through the skin and subcutis could cause technical failure and unreliable measurements[73].

Controlled attenuation parameter (CAP) is another technique implemented on the Fibroscan device. The principle of CAP is to measure the acoustic attenuation in liver of shear waves generated by the probe. The amount of fat deposited in the liver can be inferred from the degree of attenuation[74]. In a multimodality study in patients with biopsy-proven NAFLD, it was shown that using a threshold of 261 dB/m CAP the methodic accuracy was 0.85 (95% confidence interval of 0.75–0.96) for steatosis diagnosis [75].

Table 1 Pros and cons of imaging modalities to assess hepatic steatosis

Modality	Pros	Cons
US B-Mode	Lack of ionizing radiation	No panoramic view
	Less expensive	Operator dependency
	Repeatable	Limited accuracy diagnosing mild hepatic steatosis
	Fast	Rather qualitative nature
	Can be performed at the bedside (no need to transport the patient)	Non simple steatosis/NASH differentiation
	Useful also for identification of other pathology such as liver lesions	
QUS	Same as US B-Mode	Not always available
	Quantitative and semiquantitative fat evaluation (less operator sensitive)	Need to buy newer machines and software
Fibroscan	Quantitative evaluation (less operator sensitive)	Expensive equipment that doesn't supply imaging evaluation
	Lack of ionizing radiation	
	Fast	
	Can be performed at the bedside (no need to transport the patient)	
CT	Fast	Ionizing radiation
	Panoramic view	Limited accuracy diagnosing mild hepatic steatosis
	Volumetric rendering	Non simple steatosis/NASH differentiation
	High spatial resolution	
	Quantitative density evaluation	
MRI	Highly accurate and reproducible for measuring hepatic fat	Expensive
	Panoramic view	Examination time
	Lack of ionizing radiation	Software not always available
	Quantitative fat evaluation	
MRS	Highly accurate and reproducible for measuring hepatic fat	Expensive
	Panoramic view	Examination time
	Lack of ionizing radiation	Software not always available
	Quantitative fat evaluation	Evaluation of small portion of the liver
		Expertise required for data acquisition and analysis

CT: Computed tomography; MRI: Magnetic resonance imaging; MRS: Magnetic resonance spectroscopy; NASH: Nonalcoholic steatohepatitis; QUS: Quantitative ultrasound; US: Ultrasound.

Two-dimensional SWE is an US technique providing visualization of viscoelastic properties of soft tissues in real time[76]. These techniques employ acoustic radiation force impulses that induce tissue motion at a microscopic level, which in turn produces tissue shear waves. The shear waves are related to tissue stiffness under simple assumptions, expressed as Young's module[77].

In the last several years, quantitative US measures, such as the ultrasonic attenuation coefficient and backscatter coefficient, derived from the raw radiofrequency echo data, have been considered a noninvasive tool for the objective assessment of hepatic steatosis[78].

A general limitation of all US-based methods evaluating liver fat content, including CAP, is that sonography exploits the attenuation of the propagated and reflected waves. While liver fat attenuates sound waves, many other liver pathologies such as hepatitis, hemochromatosis or fibrosis can also affect sound waves in the same manner[79].

CT

CT evaluation of hepatic steatosis is based on the attenuation values of the liver parenchyma, assessed as Hounsfield units (HU), in association with tissue composition. The attenuation value of fat (approximately -100 HU) is much lower than that of soft tissue, so hepatic steatosis lowers the attenuation of liver parenchyma. Some studies have reported that contrast-enhanced venous CT and nonenhanced CT

have comparable diagnostic accuracy for hepatic steatosis[80]; however, nonenhanced CT is usually preferred to avoid the potential errors of contrast-enhanced CT caused by variations in hepatic attenuation related to contrast injection methods and scan times. The two CT indexes most frequently used to assess steatosis are the absolute liver attenuation value (*i.e.* HU-liver) and the attenuation difference between the liver and spleen.

CT is accurate for the diagnosis of moderate-to-severe steatosis but is not as accurate for detecting mild steatosis. The threshold values of CT indices for the diagnosis of hepatic steatosis are quite variable, depending on the methods and populations used[81-83]. Furthermore, some factors may affect hepatic attenuation on CT, such as the presence of excess iron in the liver and ingestion of certain drugs such as amiodarone[84].

Magnetic resonance

While CT and US assess hepatic steatosis through proxy parameters (echogenicity and attenuation, respectively), MRI can more directly measure the amount of hepatic fat, in fact it is an imaging modality with a rich range of contrast mechanisms detecting and quantifying hepatic fat content through the measurement of proton signals present in water and fat[85].

There are conventional MRI methods providing qualitative estimates of hepatic steatosis and fully quantitative MRS and MRI methods that allow for an accurate and precise measurement of hepatic fat content[86-88].

MRS and chemical shift-encoded MRI, when performed in expert hands, can serve as confounder-corrected methods able to discern the number of fat-bound protons divided by the amount of all protons in the liver, including fat- and water-bound protons[89].

To date, MRI especially with the techniques reported above, represent the noninvasive gold standard evaluation of these patients; however, US is broadly gaining popularity.

PREVENTION AND TREATMENT

NAFLD treatment depends on the severity of the disease, ranging from a more benign condition of nonalcoholic fatty liver to nonalcoholic steatohepatitis, which is at the more severe end of the spectrum. However, there are some measures that can be applied to all patients. These include the following. (1) Abstinence from alcohol: evidence shows that in NAFLD patients, there is no liver-safe limit of alcohol intake[90]. Heavy alcohol use is well-known to be associated with hepatic steatosis, hepatic injury, and progression of parenchymal fibrosis[91], but even low alcohol consumption in individuals with metabolic abnormalities could be harmful, thus abstinence from alcohol for patients with NAFLD is always recommended. (2) Immunizations: for patients without serologic evidence of immunity, vaccination for hepatitis A virus and hepatitis B virus is recommended, and, in general, standard, age-appropriate immunizations for all patients[7]. (3) Modification of risk factors for CVD: For patients with hyperlipidemia, lipid-lowering therapy; for patients with diabetes, optimizing blood glucose control[9].

For patients with NASH and T2D, the presence of the liver disease can inform the choice of glucose lowering therapy, and although this is typically with metformin, the beneficial impact on liver histology with certain other insulin-sensitizing agents could be of note when choosing a second-line agent in NASH patients, if metformin is contraindicated or in need of additional glucose-lowering therapy[33, 35]. In this setting, pioglitazone and GLP-1 receptor agonists (*e.g.*, liraglutide, semaglutide) are reasonable options[92] and the apparent benefit of certain insulin-sensitizing agents for NAFLD is likely related to the role that insulin resistance plays in the development of NAFLD[9].

For patients with biopsy-proven NASH and fibrosis stage 2 but without diabetes, the use of vitamin E (800 international units per day) is suggested. The antioxidant, anti-inflammatory, and anti-apoptotic properties of vitamin E accompanied by the ease-of-use and exceptional tolerability have made vitamin E a pragmatic therapeutic choice in nondiabetic patients with histologic evidence of NASH[93].

In every case, weight loss is the primary therapy for most patients with NAFLD. It can lead to improvement in liver biochemical tests, liver histology, serum insulin levels, and quality of life[94-96].

Several studies have suggested that weight loss of at least 5% of body weight is necessary to improve hepatic steatosis, although the long-term benefits of such weight loss are unknown. In a meta-analysis of eight trials including 373 patients, losing 5% of body weight resulted in improvement in hepatic steatosis, while losing of 7% of body weight was associated with improvement in NALFD activity score, which is used to grade disease activity[97].

Unfortunately, only less than 10% of patients that try to lose weight with lifestyle modifications, including diet and physical activity, achieve this target at 1-year, and fewer maintain the weight loss at 5 years[98]. Bariatric surgery is an option that may be considered in those who fail to lose weight by lifestyle changes.

Although weight loss seems to be the main mechanism, bariatric surgery has been shown to improve also liver histology and fibrosis secondary to NASH, in addition to other benefits including an improvement or resolution of T2D mellitus, dyslipidemia, and hypertension, and a reduction of cardiovascular morbidity or mortality[99-101].

A meta-analysis of 10 studies showed that the bariatric surgery group had significantly lower odds of major adverse cardiovascular events as compared to no surgery (odds ratio = 0.49; 95% confidence interval: 0.40-0.60; $P < 0.00001$; $I^2 = 93\%$) suggesting the benefit of bariatric surgery in reducing the occurrence of serious events in patients with obesity and CVDs[102].

In the SPLENDOR study of 1158 patients with histologically confirmed NASH and obesity, bariatric surgery (gastric bypass or sleeve gastrectomy) was associated with a much lower 10-year cumulative incidence of major adverse liver outcomes (2.3% *vs* 9.6%) and major cardiovascular events (8.5% *vs* 15.7%) compared with nonsurgical management[103].

Weight reduction due to bariatric surgery causes inflammatory changes in patients with obesity. After gastric bypass there is a proven reduction of hepatic expression of factors involved in the progression of liver inflammation (macrophage chemoattractant protein 1, and interleukin-8) and fibrogenesis [transforming growth factor-β1, tissue inhibitor of metalloproteinase 1, α-smooth muscle actin, and collagen-α1(I)][104], a significant decrease in mean NAFLD fibrosis score after Roux-en-Y gastric bypass (RYGB) and resolution rate of 55% of severe fibrosis in 12-mo observation[105], and, moreover, RYGB contributes to significant reduction in NAFLD activity score, steatosis, inflammation and liver ballooning during 1-year observation[106].

In a long-term follow-up of patients with NASH who underwent bariatric surgery, Lassailly *et al*[107] observed resolution of NASH in liver biopsies from 84% of patients 5 years later. The reduction of fibrosis is progressive, beginning during the 1st year and continuing through 5 years[107].

Among recently available surgical methods, RYGB and laparoscopic sleeve gastrectomy (LSG) are the most performed worldwide. The remaining question is whether RYGB or LSG is more effective[108].

A systematic review and meta-analysis performed by Baldwin *et al*[109] compared RYGB and LSG using separate criteria: transaminases concentration, NAFLD activity score and NAFLD fibrosis score. Overall, both RYGB and LSG significantly improved liver enzymes, NAFLD activity score, and NAFLD fibrosis score postoperatively. Direct comparisons of RYGB to LSG in any of the criteria failed to demonstrate superiority[109]. These findings, without any significant difference between the two groups, are confirmed in other studies[110,111].

Even if the role of bariatric surgery in the treatment of NAFLD is significant, there are some patients that will develop new or worsened features of NAFLD after a bariatric procedure[112]. A 5-year prospective study performed by Mathurin *et al*[113] showed that 19.8% of patients experienced fibrosis progression at 5 years follow up for unknown reason.

Aggravation of NAFLD after surgery should be kept in mind when qualifying patients for a bariatric procedure. At the extreme consequences, and when the progression of liver fibrosis is irreversible, also liver transplantation becomes an option, and indeed NASH is nowadays representing the fastest growing indication in Western countries to this kind of surgery. Yet, lifestyle modifications, as well as pharmacological strategies and tailored immunosuppression *via* a strategic multidisciplinary approach are still key to control diabetes and CVD risk in this setting, too[114].

CONCLUSION

NAFLD is intimately related to T2D and both diseases are highly prevalent worldwide, representing a public health alarm. The diagnosis and management of NAFLD in T2D is challenging, given the inherent cardiovascular risk and the underlying liver parenchymal degeneration. As well as to insulin resistance, NAFLD may be related to other hormonal alterations, quite common in patients with obesity, and potentially contributing to the onset and the worsening of steatohepatitis. A complete hormonal workout, in patients with severe NAFLD, and conversely investigation of NAFLD in patients with T2D, severe obesity or other metabolic disorders is recommended to prevent and monitor NAFLD risk.

Current medical treatments aim to mitigate insulin resistance, optimizing metabolic control and halting hepatic disease progression; yet they are still under debate for their efficacy, and new classes of drugs targeting different pathways need experimentation in the forms of randomized controlled trials, to pursue a tailor-made approach, for example assessing gut permeability and modification of individual human microbiota.

Identification of simple, inexpensive biomarkers would be also of help as an additional diagnostic tool, or to predict disease progression and response to treatment.

Surgery is considered a more advanced therapeutic option, either to improve obesity and control of the associated metabolic conditions, *via* bariatric interventions, either by substituting the cirrhotic liver *via* organ transplantation.

Future research should focus on the treatment of NAFLD, as a risk factor for developing T2D and in how to prevent and detect NAFLD progression in patients with T2D, obesity or other severe metabolic conditions.

FOOTNOTES

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REFERENCES

1 **Younossi ZM**, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, Qiu Y, Burns L, Afendy A, Nader F. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *J Hepatol* 2019; **71**: 793-801 [PMID: 31279902 DOI: 10.1016/j.jhep.2019.06.021]
2 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
3 **Le MH**, Yeo YH, Li X, Li J, Zou B, Wu Y, Ye Q, Huang DQ, Zhao C, Zhang J, Liu C, Chang N, Xing F, Yan S, Wan ZH, Tang NSY, Mayumi M, Liu X, Rui F, Yang H, Yang Y, Jin R, Le RHX, Xu Y, Le DM, Barnett S, Stave CD, Cheung R, Zhu Q, Nguyen MH. 2019 Global NAFLD Prevalence: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2021 [PMID: 34890795 DOI: 10.1016/j.cgh.2021.12.002]
4 **Loomba R**, Schork N, Chen CH, Bettencourt R, Bhatt A, Ang B, Nguyen P, Hernandez C, Richards L, Salotti J, Lin S, Seki E, Nelson KE, Sirlin CB, Brenner D; Genetics of NAFLD in Twins Consortium. Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study. *Gastroenterology* 2015; **149**: 1784-1793 [PMID: 26299412 DOI: 10.1053/j.gastro.2015.08.011]
5 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669 [PMID: 10051466 DOI: 10.1002/hep.510290347]
6 **Sheka AC**, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic Steatohepatitis: A Review. *JAMA* 2020; **323**: 1175-1183 [PMID: 32207804 DOI: 10.1001/jama.2020.2298]
7 **Li B**, Zhang C, Zhan YT. Nonalcoholic Fatty Liver Disease Cirrhosis: A Review of Its Epidemiology, Risk Factors, Clinical Presentation, Diagnosis, Management, and Prognosis. *Can J Gastroenterol Hepatol* 2018; **2018**: 2784537 [PMID: 30065915 DOI: 10.1155/2018/2784537]
8 **Buzzetti E**, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016; **65**: 1038-1048 [PMID: 26823198 DOI: 10.1016/j.metabol.2015.12.012]
9 **Gluszyńska P**, Lemancewicz D, Dzięcioł JB, Razak Hady H. Non-Alcoholic Fatty Liver Disease (NAFLD) and Bariatric/Metabolic Surgery as Its Treatment Option: A Review. *J Clin Med* 2021; **10** [PMID: 34945016 DOI: 10.3390/jcm10245721]
10 **Italian Association for the Study of the Liver (AISF)**. AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. *Dig Liver Dis* 2017; **49**: 471-483 [PMID: 28215516 DOI: 10.1016/j.dld.2017.01.147]
11 **Anstee QM**, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 330-344 [PMID: 23507799 DOI: 10.1038/nrgastro.2013.41]
12 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]
13 **Hirsova P**, Ibrabim SH, Gores GJ, Malhi H. Lipotoxic lethal and sublethal stress signaling in hepatocytes: relevance to NASH pathogenesis. *J Lipid Res* 2016; **57**: 1758-1770 [PMID: 27049024 DOI: 10.1194/jlr.R066357]
14 **Di Ciaula A**, Passarella S, Shanmugam H, Noviello M, Bonfrate L, Wang DQ, Portincasa P. Nonalcoholic Fatty Liver Disease (NAFLD). Mitochondria as Players and Targets of Therapies? *Int J Mol Sci* 2021; **22** [PMID: 34065331 DOI: 10.3390/ijms22105375]
15 **Aron-Wisnewsky J**, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, Nieuwdorp M, Clément K. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 279-297 [PMID: 32152478 DOI: 10.1038/s41575-020-0269-9]

16 **Leung C**, Rivera L, Furness JB, Angus PW. The role of the gut microbiota in NAFLD. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 412-425 [PMID: 27273168 DOI: 10.1038/nrgastro.2016.85]

17 **Di Ciaula A**, Bonfrate L, Portincasa P. The role of microbiota in nonalcoholic fatty liver disease. *Eur J Clin Invest* 2022; **52**: e13768 [PMID: 35294774 DOI: 10.1111/eci.13768]

18 **Portincasa P**, Bonfrate L, Khalil M, Angelis M, Calabrese FM, D'Amato M, Wang DQ, Di Ciaula A. Intestinal Barrier and Permeability in Health, Obesity and NAFLD. *Biomedicines* 2021; **10** [PMID: 35052763 DOI: 10.3390/biomedicines10010083]

19 **Rahman K**, Desai C, Iyer SS, Thorn NE, Kumar P, Liu Y, Smith T, Neish AS, Li H, Tan S, Wu P, Liu X, Yu Y, Farris AB, Nusrat A, Parkos CA, Anania FA. Loss of Junctional Adhesion Molecule A Promotes Severe Steatohepatitis in Mice on a Diet High in Saturated Fat, Fructose, and Cholesterol. *Gastroenterology* 2016; **151**: 733-746.e12 [PMID: 27342212 DOI: 10.1053/j.gastro.2016.06.022]

20 **Dongiovanni P**, Romeo S, Valenti L. Genetic Factors in the Pathogenesis of Nonalcoholic Fatty Liver and Steatohepatitis. *Biomed Res Int* 2015; **2015**: 460190 [PMID: 26273621 DOI: 10.1155/2015/460190]

21 **Cusi K**, Sanyal AJ, Zhang S, Hartman ML, Bue-Valleskey JM, Hoogwerf BJ, Haupt A. Non-alcoholic fatty liver disease (NAFLD) prevalence and its metabolic associations in patients with type 1 diabetes and type 2 diabetes. *Diabetes Obes Metab* 2017; **19**: 1630-1634 [PMID: 28417532 DOI: 10.1111/dom.12973]

22 **de Vries M**, Westerink J, Kaasjager KHAH, de Valk HW. Prevalence of Nonalcoholic Fatty Liver Disease (NAFLD) in Patients With Type 1 Diabetes Mellitus: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab* 2020; **105** [PMID: 32827432 DOI: 10.1210/clinem/dgaa575]

23 **Mantovani A**, Byrne CD, Bonora E, Targher G. Nonalcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: A Meta-analysis. *Diabetes Care* 2018; **41**: 372-382 [PMID: 29358469 DOI: 10.2337/dc17-1902]

24 **Gangitano E**, Ginanni Corradini S, Lubrano C, Gnessi L. La Non-Alcoholic Fatty Liver Disease, una patologia epatica di interesse endocrinologico. *L'Endocrinologo* 2021; **22**: 436-440 [DOI: 10.1007/s40619-021-00955-9]

25 **Dharmalingam M**, Yamasandhi PG. Nonalcoholic Fatty Liver Disease and Type 2 Diabetes Mellitus. *Indian J Endocrinol Metab* 2018; **22**: 421-428 [PMID: 30090738 DOI: 10.4103/ijem.IJEM_585_17]

26 **Targher G**, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus - mechanisms and treatments. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 599-612 [PMID: 33972770 DOI: 10.1038/s41575-021-00448-y]

27 **Hazlehurst JM**, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. *Metabolism* 2016; **65**: 1096-1108 [PMID: 26856933 DOI: 10.1016/j.metabol.2016.01.001]

28 **Franch-Nadal J**, Caballeria L, Mata-Cases M, Mauricio D, Giraldez-García C, Mancera J, Goday A, Mundet-Tudurí X, Regidor E; PREDAPS Study Group. Fatty liver index is a predictor of incident diabetes in patients with prediabetes: The PREDAPS study. *PLoS One* 2018; **13**: e0198327 [PMID: 29856820 DOI: 10.1371/journal.pone.0198327]

29 **Bellini MI**, Paoletti F, Herbert PE. Obesity and bariatric intervention in patients with chronic renal disease. *J Int Med Res* 2019; **47**: 2326-2341 [PMID: 31006298 DOI: 10.1177/0300060519843755]

30 **Mantovani A**, Petracca G, Beatrice G, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. *Gut* 2021; **70**: 962-969 [PMID: 32938692 DOI: 10.1136/gutjnl-2020-322572]

31 **Cuthbertson DJ**, Koskinen J, Brown E, Magnussen CG, Hutri-Kähönen N, Sabin M, Tossavainen P, Jokinen E, Laitinen T, Viikari J, Raitakari OT, Juonala M. Fatty liver index predicts incident risk of prediabetes, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD). *Ann Med* 2021; **53**: 1256-1264 [PMID: 34309471 DOI: 10.1080/07853890.2021.1956685]

32 **Kitazawa A**, Maeda S, Fukuda Y. Fatty liver index as a predictive marker for the development of diabetes: A retrospective cohort study using Japanese health check-up data. *PLoS One* 2021; **16**: e0257352 [PMID: 34543321 DOI: 10.1371/journal.pone.0257352]

33 **Ballestri S**, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, Roverato A, Guaraldi G, Lonardo A. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016; **31**: 936-944 [PMID: 26667191 DOI: 10.1111/jgh.13264]

34 **Song D**, Li C, Wang Z, Zhao Y, Shen B, Zhao W. Association of non-alcoholic fatty liver disease with diabetic retinopathy in type 2 diabetic patients: A meta-analysis of observational studies. *J Diabetes Investig* 2021; **12**: 1471-1479 [PMID: 33372390 DOI: 10.1111/jdi.13489]

35 **Mantovani A**, Dalbeni A, Beatrice G, Cappelli D, Gomez-Peralta F. Non-Alcoholic Fatty Liver Disease and Risk of Macro- and Microvascular Complications in Patients with Type 2 Diabetes. *J Clin Med* 2022; **11** [PMID: 35207239 DOI: 10.3390/jcm11040968]

36 **Lombardi R**, Airaghi L, Targher G, Serviddio G, Maffi G, Mantovani A, Maffeis C, Colecchia A, Villani R, Rinaldi L, Orsi E, Pisano G, Adinolfi LE, Fargion S, Fracanzani AL. Liver fibrosis by FibroScan® independently of established cardiovascular risk parameters associates with macrovascular and microvascular complications in patients with type 2 diabetes. *Liver Int* 2020; **40**: 347-354 [PMID: 31612634 DOI: 10.1111/liv.14274]

37 **Takahashi S**, Tanaka M, Furuhashi M, Moniwa N, Koyama M, Higashiura Y, Osanami A, Gocho Y, Ohnishi H, Numata K, Hisasue T, Hanawa N, Miura T. Fatty liver index is independently associated with deterioration of renal function during a 10-year period in healthy subjects. *Sci Rep* 2021; **11**: 8606 [PMID: 33883636 DOI: 10.1038/s41598-021-88025-w]

38 **Kouvari M**, Boutari C, Chrysohoou C, Fragkopoulou E, Antonopoulou S, Tousoulis D, Pitsavos C, Panagiotakos DB, Mantzoros CS; ATTICA study Investigators. Mediterranean diet is inversely associated with steatosis and fibrosis and decreases ten-year diabetes and cardiovascular risk in NAFLD subjects: Results from the ATTICA prospective cohort study. *Clin Nutr* 2021; **40**: 3314-3324 [PMID: 33234342 DOI: 10.1016/j.clnu.2020.10.058]

39 **Watanabe M**, Tozzi R, Risi R, Tuccinardi D, Mariani S, Basciani S, Spera G, Lubrano C, Gnessi L. Beneficial effects of the ketogenic diet on nonalcoholic fatty liver disease: A comprehensive review of the literature. *Obes Rev* 2020; **21**:

e13024 [PMID: 32207237 DOI: 10.1111/obr.13024]
40 **GBD 2017 Causes of Death Collaborators**. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; **392**: 1736-1788 [PMID: 30496103 DOI: 10.1016/S0140-6736(18)32203-7]
41 **American Diabetes Association**. . 10. Cardiovascular Disease and Risk Management: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020; **43**: S111-S134 [PMID: 31862753 DOI: 10.2337/dc20-S010]
42 **Han AL**. Association of Cardiovascular Risk Factors and Metabolic Syndrome with non-alcoholic and alcoholic fatty liver disease: a retrospective analysis. *BMC Endocr Disord* 2021; **21**: 91 [PMID: 33933056 DOI: 10.1186/s12902-021-00758-x]
43 **Kasper P**, Martin A, Lang S, Kütting F, Goeser T, Demir M, Steffen HM. NAFLD and cardiovascular diseases: a clinical review. *Clin Res Cardiol* 2021; **110**: 921-937 [PMID: 32696080 DOI: 10.1007/s00392-020-01709-7]
44 **Mantovani A**, Csermely A, Petracca G, Beatrice G, Corey KE, Simon TG, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of fatal and non-fatal cardiovascular events: an updated systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2021; **6**: 903-913 [PMID: 34555346 DOI: 10.1016/S2468-1253(21)00308-3]
45 **Higashiura Y**, Furuhashi M, Tanaka M, Takahashi S, Mori K, Miyamori D, Koyama M, Ohnishi H, Moniwa N, Numata K, Hisasue T, Hanawa N, Miura T. Elevated Fatty Liver Index Is Independently Associated With New Onset of Hypertension During a 10-Year Period in Both Male and Female Subjects. *J Am Heart Assoc* 2021; **10**: e021430 [PMID: 34259033 DOI: 10.1161/JAHA.121.021430]
46 **Park J**, Kim G, Kim H, Lee J, Lee YB, Jin SM, Hur KY, Kim JH. The association of hepatic steatosis and fibrosis with heart failure and mortality. *Cardiovasc Diabetol* 2021; **20**: 197 [PMID: 34583706 DOI: 10.1186/s12933-021-01374-8]
47 **Lee CH**, Han KD, Kim DH, Kwak MS. The Repeatedly Elevated Fatty Liver Index Is Associated With Increased Mortality: A Population-Based Cohort Study. *Front Endocrinol (Lausanne)* 2021; **12**: 638615 [PMID: 33776934 DOI: 10.3389/fendo.2021.638615]
48 **Caussy C**, Aubin A, Loomba R. The Relationship Between Type 2 Diabetes, NAFLD, and Cardiovascular Risk. *Curr Diab Rep* 2021; **21**: 15 [PMID: 33742318 DOI: 10.1007/s11892-021-01383-7]
49 **Wild SH**, Walker JJ, Morling JR, McAllister DA, Colhoun HM, Farran B, McGurnaghan S, McCrimmon R, Read SH, Sattar N, Byrne CD; Scottish Diabetes Research Network Epidemiology Group. Cardiovascular Disease, Cancer, and Mortality Among People With Type 2 Diabetes and Alcoholic or Nonalcoholic Fatty Liver Disease Hospital Admission. *Diabetes Care* 2018; **41**: 341-347 [PMID: 29167212 DOI: 10.2337/dc17-1590]
50 **Cavender MA**, Steg PG, Smith SC Jr, Eagle K, Ohman EM, Goto S, Kuder J, Im K, Wilson PW, Bhatt DL; REACH Registry Investigators. Impact of Diabetes Mellitus on Hospitalization for Heart Failure, Cardiovascular Events, and Death: Outcomes at 4 Years From the Reduction of Atherothrombosis for Continued Health (REACH) Registry. *Circulation* 2015; **132**: 923-931 [PMID: 26152709 DOI: 10.1161/CIRCULATIONAHA.114.014796]
51 **Targher G**, Pichiri I, Zoppini G, Trombetta M, Bonora E. Increased prevalence of chronic kidney disease in patients with Type 1 diabetes and non-alcoholic fatty liver. *Diabet Med* 2012; **29**: 220-226 [PMID: 21883436 DOI: 10.1111/j.1464-5491.2011.03427.x]
52 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020; **323**: 2052-2059 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]
53 **Hussain S**, Baxi H, Chand Jamali M, Nisar N, Hussain MS. Burden of diabetes mellitus and its impact on COVID-19 patients: A meta-analysis of real-world evidence. *Diabetes Metab Syndr* 2020; **14**: 1595-1602 [PMID: 32862098 DOI: 10.1016/j.dsx.2020.08.014]
54 **Suleyman G**, Fadel RA, Malette KM, Hammond C, Abdulla H, Entz A, Demertzis Z, Hanna Z, Failla A, Dagher C, Chaudhry Z, Vahia A, Abreu Lanfranco O, Ramesh M, Zervos MJ, Alangaden G, Miller J, Brar I. Clinical Characteristics and Morbidity Associated With Coronavirus Disease 2019 in a Series of Patients in Metropolitan Detroit. *JAMA Netw Open* 2020; **3**: e2012270 [PMID: 32543702 DOI: 10.1001/jamanetworkopen.2020.12270]
55 **Roncon L**, Zuin M, Rigatelli G, Zuliani G. Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome. *J Clin Virol* 2020; **127**: 104354 [PMID: 32305882 DOI: 10.1016/j.jcv.2020.104354]
56 **Miller LE**, Bhattacharyya R, Miller AL. Diabetes mellitus increases the risk of hospital mortality in patients with Covid-19: Systematic review with meta-analysis. *Medicine (Baltimore)* 2020; **99**: e22439 [PMID: 33019426 DOI: 10.1097/MD.0000000000022439]
57 **Dongiovanni P**, Meroni M, Longo M, Fracanzani AL. MAFLD in COVID-19 patients: an insidious enemy. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 867-872 [PMID: 32705906 DOI: 10.1080/17474124.2020.1801417]
58 **Singh A**, Hussain S, Antony B. Non-alcoholic fatty liver disease and clinical outcomes in patients with COVID-19: A comprehensive systematic review and meta-analysis. *Diabetes Metab Syndr* 2021; **15**: 813-822 [PMID: 33862417 DOI: 10.1016/j.dsx.2021.03.019]
59 **Trivedi HD**, Wilechansky R, Goyes D, Vieira Barbosa J, Canakis A, Lai M, Long MT, Fricker Z. Radiographic Hepatic Steatosis Is Not Associated With Key Clinical Outcomes Among Patients Hospitalized With COVID-19. *Gastroenterology Res* 2021; **14**: 179-183 [PMID: 34267833 DOI: 10.14740/gr1389]
60 **Li J**, Tian A, Zhu H, Chen L, Wen J, Liu W, Chen P. Mendelian Randomization Analysis Reveals No Causal Relationship Between Nonalcoholic Fatty Liver Disease and Severe COVID-19. *Clin Gastroenterol Hepatol* 2022; **20**: 1553-1560.e78 [PMID: 35124268 DOI: 10.1016/j.cgh.2022.01.045]
61 **Forlano R**, Mullish BH, Mukherjee SK, Nathwani R, Harlow C, Crook P, Judge R, Soubieres A, Middleton P, Daunt A, Perez-Guzman P, Selvapatt N, Lemoine M, Dhar A, Thursz MR, Nayagam S, Manousou P. In-hospital mortality is associated with inflammatory response in NAFLD patients admitted for COVID-19. *PLoS One* 2020; **15**: e0240400 [PMID: 33031439 DOI: 10.1371/journal.pone.0240400]

62 **Ji D**, Qin E, Xu J, Zhang D, Cheng G, Wang Y, Lau G. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020; **73**: 451-453 [PMID: 32278005 DOI: 10.1016/j.jhep.2020.03.044]

63 **Targher G**, Mantovani A, Byrne CD, Wang XB, Yan HD, Sun QF, Pan KH, Zheng KI, Chen YP, Eslam M, George J, Zheng MH. Risk of severe illness from COVID-19 in patients with metabolic dysfunction-associated fatty liver disease and increased fibrosis scores. *Gut* 2020; **69**: 1545-1547 [PMID: 32414813 DOI: 10.1136/gutjnl-2020-321611]

64 **Buckley AJ**, Thomas EL, Lessan N, Trovato FM, Trovato GM, Taylor-Robinson SD. Non-alcoholic fatty liver disease: Relationship with cardiovascular risk markers and clinical endpoints. *Diabetes Res Clin Pract* 2018; **144**: 144-152 [PMID: 30170074 DOI: 10.1016/j.diabres.2018.08.011]

65 **Idilman IS**, Aniktar H, Idilman R, Kabacam G, Savas B, Elhan A, Celik A, Bahar K, Karcaaltincaba M. Hepatic steatosis: quantification by proton density fat fraction with MR imaging versus liver biopsy. *Radiology* 2013; **267**: 767-775 [PMID: 23382293 DOI: 10.1148/radiol.13121360]

66 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

67 **Ferraioli G**, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. *World J Gastroenterol* 2019; **25**: 6053-6062 [PMID: 31686762 DOI: 10.3748/wjg.v25.i40.6053]

68 **Hernaez R**, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; **54**: 1082-1090 [PMID: 21618575 DOI: 10.1002/hep.24452]

69 **Singh S**, Venkatesh SK, Loomba R, Wang Z, Sirlin C, Chen J, Yin M, Miller FH, Low RN, Hassanein T, Godfrey EM, Asbach P, Murad MH, Lomas DJ, Talwalkar JA, Ehman RL. Magnetic resonance elastography for staging liver fibrosis in non-alcoholic fatty liver disease: a diagnostic accuracy systematic review and individual participant data pooled analysis. *Eur Radiol* 2016; **26**: 1431-1440 [PMID: 26314479 DOI: 10.1007/s00330-015-3949-z]

70 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745 DOI: 10.1002/hep.23312]

71 **Yoneda M**, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. *Radiology* 2010; **256**: 640-647 [PMID: 20529989 DOI: 10.1148/radiol.10091662]

72 **Chen J**, Talwalkar JA, Yin M, Glaser KJ, Sanderson SO, Ehman RL. Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. *Radiology* 2011; **259**: 749-756 [PMID: 21460032 DOI: 10.1148/radiol.11101942]

73 **Dyson JK**, McPherson S, Anstee QM. Republished: Non-alcoholic fatty liver disease: non-invasive investigation and risk stratification. *Postgrad Med J* 2014; **90**: 254-266 [PMID: 24737902 DOI: 10.1136/postgradmedj-2013-201620rep]

74 **Vuppalanchi R**, Siddiqui MS, Van Natta ML, Hallinan E, Brandman D, Kowdley K, Neuschwander-Tetri BA, Loomba R, Dasarathy S, Abdelmalek M, Doo E, Tonascia JA, Kleiner DE, Sanyal AJ, Chalasani N; NASH Clinical Research Network. Performance characteristics of vibration-controlled transient elastography for evaluation of nonalcoholic fatty liver disease. *Hepatology* 2018; **67**: 134-144 [PMID: 28859228 DOI: 10.1002/hep.29489]

75 **Park CC**, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, Hooker J, Sy E, Savides MT, Alquiraish MH, Valasek MA, Rizo E, Richards L, Brenner D, Sirlin CB, Loomba R. Magnetic Resonance Elastography vs Transient Elastography in Detection of Fibrosis and Noninvasive Measurement of Steatosis in Patients With Biopsy-Proven Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2017; **152**: 598-607.e2 [PMID: 27911262 DOI: 10.1053/j.gastro.2016.10.026]

76 **Bercoff J**, Tanter M, Fink M. Supersonic shear imaging: a new technique for soft tissue elasticity mapping. *IEEE Trans Ultrason Ferroelectr Freq Control* 2004; **51**: 396-409 [PMID: 15139541 DOI: 10.1109/tuffc.2004.1295425]

77 **Bota S**, Sporea I, Peck-Radosavljevic M, Sirli R, Tanaka H, Iijima H, Saito H, Ebinuma H, Lupsor M, Badea R, Fierbinteanu-Braticevici C, Petrisor A, Friedrich-Rust M, Sarrazin C, Takahashi H, Ono N, Piscaglia F, Marinelli S, D'Onofrio M, Gallotti A, Salzl P, Popescu A, Danila M. The influence of aminotransferase levels on liver stiffness assessed by Acoustic Radiation Force Impulse Elastography: a retrospective multicentre study. *Dig Liver Dis* 2013; **45**: 762-768 [PMID: 23510533 DOI: 10.1016/j.dld.2013.02.008]

78 **Jeon SK**, Lee JM, Joo I. Clinical Feasibility of Quantitative Ultrasound Imaging for Suspected Hepatic Steatosis: Intra- and Inter-examiner Reliability and Correlation with Controlled Attenuation Parameter. *Ultrasound Med Biol* 2021; **47**: 438-445 [PMID: 33277108 DOI: 10.1016/j.ultrasmedbio.2020.11.009]

79 **Siegelman ES**. MR imaging of diffuse liver disease. Hepatic fat and iron. *Magn Reson Imaging Clin N Am* 1997; **5**: 347-365 [PMID: 9113679]

80 **Kim DY**, Park SH, Lee SS, Kim HJ, Kim SY, Kim MY, Lee Y, Kim TK, Khalili K, Bae MH, Lee JY, Lee SG, Yu ES. Contrast-enhanced computed tomography for the diagnosis of fatty liver: prospective study with same-day biopsy used as the reference standard. *Eur Radiol* 2010; **20**: 359-366 [PMID: 19727755 DOI: 10.1007/s00330-009-1560-x]

81 **Lee SS**, Park SH, Kim HJ, Kim SY, Kim MY, Kim DY, Suh DJ, Kim KM, Bae MH, Lee JY, Lee SG, Yu ES. Non-invasive assessment of hepatic steatosis: prospective comparison of the accuracy of imaging examinations. *J Hepatol* 2010; **52**: 579-585 [PMID: 20185194 DOI: 10.1016/j.jhep.2010.01.008]

82 **van Werven JR**, Marsman HA, Nederveen AJ, Smits NJ, ten Kate FJ, van Gulik TM, Stoker J. Assessment of hepatic steatosis in patients undergoing liver resection: comparison of US, CT, T1-weighted dual-echo MR imaging, and point-resolved 1H MR spectroscopy. *Radiology* 2010; **256**: 159-168 [PMID: 20574093 DOI: 10.1148/radiol.10091790]

83 **Hepburn MJ**, Vos JA, Fillman EP, Lawitz EJ. The accuracy of the report of hepatic steatosis on ultrasonography in patients infected with hepatitis C in a clinical setting: a retrospective observational study. *BMC Gastroenterol* 2005; **5**: 14 [PMID: 15829009 DOI: 10.1186/1471-230X-5-14]

84 **Lv HJ**, Zhao HW. Amiodarone-induced hepatotoxicity - quantitative measurement of iodine density in the liver using

dual-energy computed tomography: Three case reports. *World J Clin Cases* 2020; **8**: 4958-4965 [PMID: 33195667 DOI: 10.12998/wjcc.v8.i20.4958]

85 **Kramer H**, Pickhardt PJ, Kliewer MA, Hernando D, Chen GH, Zagzebski JA, Reeder SB. Accuracy of Liver Fat Quantification With Advanced CT, MRI, and Ultrasound Techniques: Prospective Comparison With MR Spectroscopy. *AJR Am J Roentgenol* 2017; **208**: 92-100 [PMID: 27726414 DOI: 10.2214/AJR.16.16565]

86 **Benedict M**, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol* 2017; **9**: 715-732 [PMID: 28652891 DOI: 10.4254/wjh.v9.i16.715]

87 **Caussy C**, Reeder SB, Sirlin CB, Loomba R. Noninvasive, Quantitative Assessment of Liver Fat by MRI-PDFF as an Endpoint in NASH Trials. *Hepatology* 2018; **68**: 763-772 [PMID: 29356032 DOI: 10.1002/hep.29797]

88 **Yokoo T**, Serai SD, Pirasteh A, Bashir MR, Hamilton G, Hernando D, Hu HH, Hetterich H, Kühn JP, Kukuk GM, Loomba R, Middleton MS, Obuchowski NA, Song JS, Tang A, Wu X, Reeder SB, Sirlin CB; RSNA-QIBA PDFF Biomarker Committee. Linearity, Bias, and Precision of Hepatic Proton Density Fat Fraction Measurements by Using MR Imaging: A Meta-Analysis. *Radiology* 2018; **286**: 486-498 [PMID: 28892458 DOI: 10.1148/radiol.2017170550]

89 **Reeder SB**, Hu HH, Sirlin CB. Proton density fat-fraction: a standardized MR-based biomarker of tissue fat concentration. *J Magn Reson Imaging* 2012; **36**: 1011-1014 [PMID: 22777847 DOI: 10.1002/jmri.23741]

90 **Di Ciaula A**, Bonfrate L, Krawczyk M, Frühbeck G, Portincasa P. Synergistic and Detrimental Effects of Alcohol Intake on Progression of Liver Steatosis. *Int J Mol Sci* 2022; **23** [PMID: 35269779 DOI: 10.3390/ijms23052636]

91 **Ekstedt M**, Franzén LE, Holmqvist M, Bendtsen P, Mathiesen UL, Bodemar G, Kechagias S. Alcohol consumption is associated with progression of hepatic fibrosis in non-alcoholic fatty liver disease. *Scand J Gastroenterol* 2009; **44**: 366-374 [PMID: 19016382 DOI: 10.1080/00365520802555991]

92 **Boettcher E**, Csako G, Pucino F, Wesley R, Loomba R. Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2012; **35**: 66-75 [PMID: 22050199 DOI: 10.1111/j.1365-2036.2011.04912.x]

93 **Perumpail BJ**, Li AA, John N, Sallam S, Shah ND, Kwong W, Cholankeril G, Kim D, Ahmed A. The Role of Vitamin E in the Treatment of NAFLD. *Diseases* 2018; **6** [PMID: 30249972 DOI: 10.3390/diseases6040086]

94 **Vilar-Gomez E**, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, Diago M, Romero-Gomez M. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015; **149**: 367-78.e5; quiz e14 [PMID: 25865049 DOI: 10.1053/j.gastro.2015.04.005]

95 **Promrat K**, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, Wing RR. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 121-129 [PMID: 19827166 DOI: 10.1002/hep.23276]

96 **Keating SE**, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012; **57**: 157-166 [PMID: 22414768 DOI: 10.1016/j.jhep.2012.02.023]

97 **Musso G**, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia* 2012; **55**: 885-904 [PMID: 22278337 DOI: 10.1007/s00125-011-2446-4]

98 **Wing RR**, Phelan S. Long-term weight loss maintenance. *Am J Clin Nutr* 2005; **82**: 222S-225S [PMID: 16002825 DOI: 10.1093/ajcn/82.1.222S]

99 **Ha J**, Jang M, Kwon Y, Park YS, Park DJ, Lee JH, Lee HJ, Ha TK, Kim YJ, Han SM, Han SU, Heo Y, Park S. Metabolomic Profiles Predict Diabetes Remission after Bariatric Surgery. *J Clin Med* 2020; **9** [PMID: 33271740 DOI: 10.3390/jcm9123897]

100 **Diemieszczyk I**, Woźniewska P, Gołaszewski P, Drygalski K, Nadolny K, Ładny JR, Razak Hady H. Does weight loss after laparoscopic sleeve gastrectomy contribute to reduction in blood pressure? *Pol Arch Intern Med* 2021; **131**: 693-700 [PMID: 34075736 DOI: 10.20452/pamw.16023]

101 **Doumouras AG**, Wong JA, Paterson JM, Lee Y, Sivapathasundaram B, Tarride JE, Thabane L, Hong D, Yusuf S, Anvari M. Bariatric Surgery and Cardiovascular Outcomes in Patients With Obesity and Cardiovascular Disease:: A Population-Based Retrospective Cohort Study. *Circulation* 2021; **143**: 1468-1480 [PMID: 33813836 DOI: 10.1161/CIRCULATIONAHA.120.052386]

102 **Sutanto A**, Wungu CDK, Susilo H, Sutanto H. Reduction of Major Adverse Cardiovascular Events (MACE) after Bariatric Surgery in Patients with Obesity and Cardiovascular Diseases: A Systematic Review and Meta-Analysis. *Nutrients* 2021; **13** [PMID: 34684569 DOI: 10.3390/nu13103568]

103 **Aminian A**, Al-Kurd A, Wilson R, Bena J, Fayazzadeh H, Singh T, Albaugh VL, Shariff FU, Rodriguez NA, Jin J, Brethauer SA, Dasarathy S, Alkhouri N, Schauer PR, McCullough AJ, Nissen SE. Association of Bariatric Surgery With Major Adverse Liver and Cardiovascular Outcomes in Patients With Biopsy-Proven Nonalcoholic Steatohepatitis. *JAMA* 2021; **326**: 2031-2042 [PMID: 34762106 DOI: 10.1001/jama.2021.19569]

104 **Klein S**, Mittendorfer B, Eagon JC, Patterson B, Grant L, Feirt N, Seki E, Brenner D, Korenblat K, McCrea J. Gastric bypass surgery improves metabolic and hepatic abnormalities associated with nonalcoholic fatty liver disease. *Gastroenterology* 2006; **130**: 1564-1572 [PMID: 16697719 DOI: 10.1053/j.gastro.2006.01.042]

105 **Cazzo E**, Jimenez LS, Pareja JC, Chaim EA. Effect of Roux-en-Y gastric bypass on nonalcoholic fatty liver disease evaluated through NAFLD fibrosis score: a prospective study. *Obes Surg* 2015; **25**: 982-985 [PMID: 25381118 DOI: 10.1007/s11695-014-1489-2]

106 **Pedersen JS**, Rygg MO, Serizawa RR, Kristiansen VB, Albrechtsen NJW, Gluud LL, Madsbad S, Bendtsen F. Effects of Roux-en-Y Gastric Bypass and Sleeve Gastrectomy on Non-Alcoholic Fatty Liver Disease: A 12-Month Follow-Up Study with Paired Liver Biopsies. *J Clin Med* 2021; **10** [PMID: 34501231 DOI: 10.3390/jcm10173783]

107 **Lassailly G**, Caiazzo R, Ntandja-Wandji LC, Gnemmi V, Baud G, Verkindt H, Ningarhari M, Louvet A, Leteurtre E, Raverdy V, Dharancy S, Pattou F, Mathurin P. Bariatric Surgery Provides Long-term Resolution of Nonalcoholic Steatohepatitis and Regression of Fibrosis. *Gastroenterology* 2020; **159**: 1290-1301.e5 [PMID: 32553765 DOI: 10.1053/j.gastro.2020.06.006]

108 **Cerci M**, Bellini MI, Russo F, Benavoli D, Capperucci M, Gaspari AL, Gentileschi P. Bariatric surgery in moderately obese patients: a prospective study. *Gastroenterol Res Pract* 2013; **2013**: 276183 [PMID: 24454338 DOI: 10.1155/2013/276183]

109 **Baldwin D**, Chennakesavalu M, Gangemi A. Systematic review and meta-analysis of Roux-en-Y gastric bypass against laparoscopic sleeve gastrectomy for amelioration of NAFLD using four criteria. *Surg Obes Relat Dis* 2019; **15**: 2123-2130 [PMID: 31711944 DOI: 10.1016/j.soard.2019.09.060]

110 **de Brito E Silva MB**, Tustumi F, de Miranda Neto AA, Dantas ACB, Santo MA, Cecconello I. Gastric Bypass Compared with Sleeve Gastrectomy for Nonalcoholic Fatty Liver Disease: a Systematic Review and Meta-analysis. *Obes Surg* 2021; **31**: 2762-2772 [PMID: 33846949 DOI: 10.1007/s11695-021-05412-y]

111 **Cherla DV**, Rodriguez NA, Vangoitsenhoven R, Singh T, Mehta N, McCullough AJ, Brethauer SA, Schauer PR, Aminian A. Impact of sleeve gastrectomy and Roux-en-Y gastric bypass on biopsy-proven non-alcoholic fatty liver disease. *Surg Endosc* 2020; **34**: 2266-2272 [PMID: 31359195 DOI: 10.1007/s00464-019-07017-0]

112 **Lee Y**, Doumouras AG, Yu J, Brar K, Banfield L, Gmora S, Anvari M, Hong D. Complete Resolution of Nonalcoholic Fatty Liver Disease After Bariatric Surgery: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2019; **17**: 1040-1060.e11 [PMID: 30326299 DOI: 10.1016/j.cgh.2018.10.017]

113 **Mathurin P**, Hollebecque A, Arnalsteen L, Buob D, Leteurtre E, Caiazzo R, Pigeyre M, Verkindt H, Dharancy S, Louvet A, Romon M, Pattou F. Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. *Gastroenterology* 2009; **137**: 532-540 [PMID: 19409898 DOI: 10.1053/j.gastro.2009.04.052]

114 **Burra P**, Becchetti C, Germani G. NAFLD and liver transplantation: Disease burden, current management and future challenges. *JHEP Rep* 2020; **2**: 100192 [PMID: 33163950 DOI: 10.1016/j.jhepr.2020.100192]

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REVIEW

A review of potential mechanisms and uses of SGLT2 inhibitors in ischemia-reperfusion phenomena

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Abstract

Recently added to the therapeutic arsenal against chronic heart failure as a first intention drug, the antidiabetic drug-class sodium-glucose cotransporter-2 inhibitors (SGLT2i) showed efficacy in decreasing overall mortality, hospitalization, and sudden death in patients of this very population, in whom chronic or acute ischemia count among the first cause. Remarkably, this benefit was observed independently from diabetic status, and benefited both preserved and altered ventricular ejection fraction. This feature, observed in several large randomized controlled trials, suggests additional effects from SGLT2i beyond isolated glycemia control. Indeed, both in-vitro and animal models suggest that inhibiting the Na^+/H^+ exchanger (NHE) may be key to preventing ischemia/reperfusion injuries, and by extension may hold a similar role in ischemic damage control and ischemic preconditioning. Yet, several other mechanisms may be explored which may help better target those who may benefit most from SGLT2i molecules. Because of a large therapeutic margin with few adverse events, ease of prescription and potential pharmacological efficacity, SGLT2i could be candidate for wider indications. In this review, we aim to summarize all evidence which link SGLT2i and ischemia/reperfusion injuries modulation, by first listing known mechanisms, including metabolic switch, prevention of lethal arrythmias and others, which portend the latter, and second, hypothesize how the former may interact with these mechanisms.

Key Words: SGLT2 inhibitors; Ischemia-reperfusion injuries; Sodium-proton exchanger; Myocardial ischemia; Immunomodulation

Core Tip: The antidiabetic drug-class sodium-glucose cotransporter-2 inhibitors (SGLT2i) showed efficacy in decreasing mortality in patients with chronic heart failure, in whom ischemia counts among the first cause. Remarkably, this benefit was observed independently from diabetic status. This feature, yielded from several randomized controlled trials, suggests additional effects from SGLT2i beyond isolated glycemia control. Indeed, previous in-vitro and animal models analyzed altogether suggests the role of the inhibition of the Na^+/H^+ exchanger, which holds a pivotal role in ischemia/reperfusion injuries. In this review, we aim to summarize evidence which associate SGLT2i and ischemia/reperfusion injuries, by first listing known mechanisms which portend the latter, and second, hypothesize how the former may interact with these mechanisms.

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INTRODUCTION

Although sodium-glucose cotransporter-2 inhibitors (SGLT2i) represent a decade-old drug class, the range of their indications has expanded since the first Food and Drug Administration label in 2013 in patients with type 2 diabetes[1,2]. Indeed, SGLT2i which include empagliflozin, dapagliflozin and canagliflozin are now indicated in patients with heart failure, independently from their status towards diabetes[3].

To understand how SGLT2i went from an antidiabetic to a cardioprotective treatment, one must recall how in patients with type 2 diabetes treated by SGLT2i, there were numerous observations of a decrease in heart failure events, all-cause mortality, cardiovascular mortality[4]. Furthermore, subgroup analyses confirmed that this risk decrease was consistent across a wide range of cardiovascular risk[5,6].

Hence, specific randomized controlled trials were launched to assess the hypothesis of a benefit to be treated by SGLT2i for patients with heart failure, regardless of the presence or absence of diabetes. Preliminary reports were then confirmed, and SGLT2i improved clinical outcomes in patients presenting with heart failure, be they with preserved and reduced ejection fraction[2,7-9].

Nevertheless, while the main pharmacological effect of SGLT2i is to decrease renal glucose reabsorption, thereby increasing urinary glucose excretion, the benefits observed even in non-diabetic patients question off-target mechanisms. As an illustration, in the EMPA-REG OUTCOME trial which compared empagliflozin to placebo in patients with type 2 diabetes at high risk for cardiovascular events, the proportion of acute myocardial or cerebral ischemic event was similar in both groups, however, patients in the treatment group were more likely to surviving a cardiovascular event. This element may be supportive of a cellular protective association in ischemic injury[10]. In the dapagliflozin and prevention of adverse-outcomes in heart failure trial (DAPA-HF), administration of dapagliflozin reduced risk of serious ventricular arrythmia, cardiac arrest or sudden death[11].

In the following review, we aimed to suggest several mechanisms which may explain how SGLT2i act as immunomodulators, and how they may act beyond the sole increase in urinary loss of glucose. We first described the ischemia-reperfusion injury phenomenon and then expanded on the interactions between SGLT2i and ischemia-reperfusion mechanisms. Our main assumption lied on a protective role against ischemia-reperfusion lesions, which involve an increase in functional ketones, associated with a metabolic change, an impact on sodium/hydrogen exchanger, endothelial dysfunction, inflammation biomarkers, and platelet function.

ISCHEMIA-REPERFUSION INJURY, AN OVERVIEW

While mortality of acute myocardial infarction, has been decreasing over time[12], subsequent morbidity manifested by heart failure has grown. Mitigating infarct size is a therapeutic goal which may be attained by decreasing the delay between first signs of ischemia and revascularization[13], and by managing secondary lesions.

Myocardial ischemia is often caused by the occlusion of epicardial artery resulting in the ischemia of the coronary vascular territory which it depends upon. If prolonged, it may lead to myocardial infarction, an irreversible condition[14,15]. Therefore, quickly restoring blood flow in the occluded artery is the only way to limit the extent of infarction and subsequent complications including mortality. The reperfusion phenomenon however has been associated with secondary lesions[16], responsible for additional cardiomyocyte injuries[17,18]. These additional lesions may be partly responsible of final infarction size and therefore associated with adverse outcomes as there is a link between infraction size

and long-term mortality or heart failure[19].

In cardiac surgery, these lesions are detected in 25% to 45% of patients[20]. They may be assessed by CK-MB and/or troponin levels, associated with postoperative adverse events[21]: arrythmias, myocardial stunning, low cardiac output syndrome and perioperative infarction[22]. Although, situations leading to these myocardial injuries are either unpredictable (*i.e.*, acute myocardial infarction) or unavoidable (*i.e.*, cardiac surgery), cardioprotective strategies aiming at reducing ischemia/reperfusion injury are critical[23].

Myocardial ischemia

Defined by a mismatch between supply and need in oxygen and nutrients, its consequences depend on its severity, duration and the existence of collateral circulation[24]. In normal blood flow situation, oxygen is used by mitochondrial respiratory chain to produce ATP by using fatty acids (65%), glucose (15%), lactate (15%) and amino-acids and ketones (5%). Ninety percent of produced ATP are used by cardiomyocytes for contraction and the rest for homeostasis[25]. Following arterial occlusion and oxygen supply arrest, oxidative phosphorylation by mitochondrial respiratory chain stops and metabolism becomes anaerobic with the use of anaerobic glycogenolysis, leading to formation of H^+ and lactates[26]. Hence, during ischemia, ATP is mainly produced from glucose instead of fatty acids, due to a higher energy-consumption rate of fatty acids catabolism[27]. This metabolic shift leads to the accumulation of AcylCoA and AcylCarnitine, both considered toxic for cardiomyocytes (enzymatic inhibition, alters cell membrane etc.). The small amount of produced ATP is used to maintain cellular homeostasis by using ATP-dependent ion pumps, until all ATP are depleted. Owing to ATP deficiency some cellular functions are not further ensured such as myocardial contraction, protein synthesis[28].

Then, an intracellular sodium accumulation creates a cellular oedema due to the activation of Na^+/H^+ exchanger (NHE) and inhibition of NA/K ATPase, which in turn, leads to a cytosolic calcium overload by activation of Na^+/Ca^{2+} exchanger[29,30], inhibition of SERCA[30], and increased calcium entry *via* other channels[30].

The subsequent activation of protease, lipase, nuclease[27], and mitochondrial ultrastructural damage, are associated with myocardial stunning. In normal conditions, mitochondria's membrane is impermeable to ions and proteins[22], with a channel on the inner membrane called the mitochondrial permeability transition pore (mPTP)[25]. During ischemia, this permeability transitions, opening mPTP [22], leading to mitochondrial oedema and death and release of its contents: Cytochrome c, apoptosis-inducing factor AIF, reactive oxygen species (ROS)[31,32].

ROS are highly reactive elements responsible for cellular injury because of reactions with lipids, proteins, and nucleic acids. The accumulation of xanthine and hypoxantine during ischemia[33], allows for their use by xanthine oxidase, activated during reperfusion and leading to the formation of ROS[34]. One of the many sources of hypoxanthine during ischemia, is ATP degradation by adenine nucleotide translocase which synthesize ADP, then degraded into hypoxanthine. This phenomenon increases energetic deficiency.

Reperfusion injury

After myocardial ischemia, restoring blood flow is an emergency, and clinical guidelines all advocate for the shortest delay possible[13,25]. However, reperfusion is also associated with secondary injuries[35], due to the sudden oxygen supply which allows for the formation of superoxide anions. The mechanisms which are hypothesized include: (1) The activation of oxidative phosphorylation; (2) the activation of xanthine oxidase; and (3) local neutrophil accumulation and NADPH oxidase activation, also leading to ROS accumulation[25]. In normal conditions, superoxide anions are antagonized by antioxidant elements (catalase, superoxide dismutase, glutathione peroxidase, vitamins, *etc*.). However, in case of massive ROS production and altered defense mechanisms by ischemia, the balance is tipped off towards ROS accumulation. A graphic summary of these mechanisms is available in Figure 1.

Another mechanism of reperfusion injury is the pH paradox[25,36]**.** Reperfusion restores pH by quickly extracting accumulated H^+, by activating of NHE; yet, pH restoration has been associated with deleterious outcomes[37]. Indeed, an abrupt accumulation of Na^+ may lead to cellular oedema and calcium overload (due to a Na^+/Ca^{2+} exchanger), and since cytoplasmic acidosis inhibits the mPTP opening, rapid normalization of intracellular pH leads to mitochondrial permeability transition with mPTP reopening[27]. Hence, phenomena similar to that of ischemia may occur even though reperfusion was achieved[29].

Cardioprotective strategies

Cardioprotective strategies aim to reduce cardiomyocytes injuries, secondary to ischemia-reperfusion phenomena, and include 4 methods: preconditioning, postconditioning, remote conditioning and pharmacological treatment.

Preconditioning consists in applying cycles of brief coronary occlusion immediately before sustained occlusion. Clinical benefit has been observed in dog models, where repetitive short coronary occlusions preceding sustained occlusion resulted with an infarction smaller more delayed than that of a sustained occlusion without preconditioning[38]. While the benefit was initially observed shortly after ischemia,

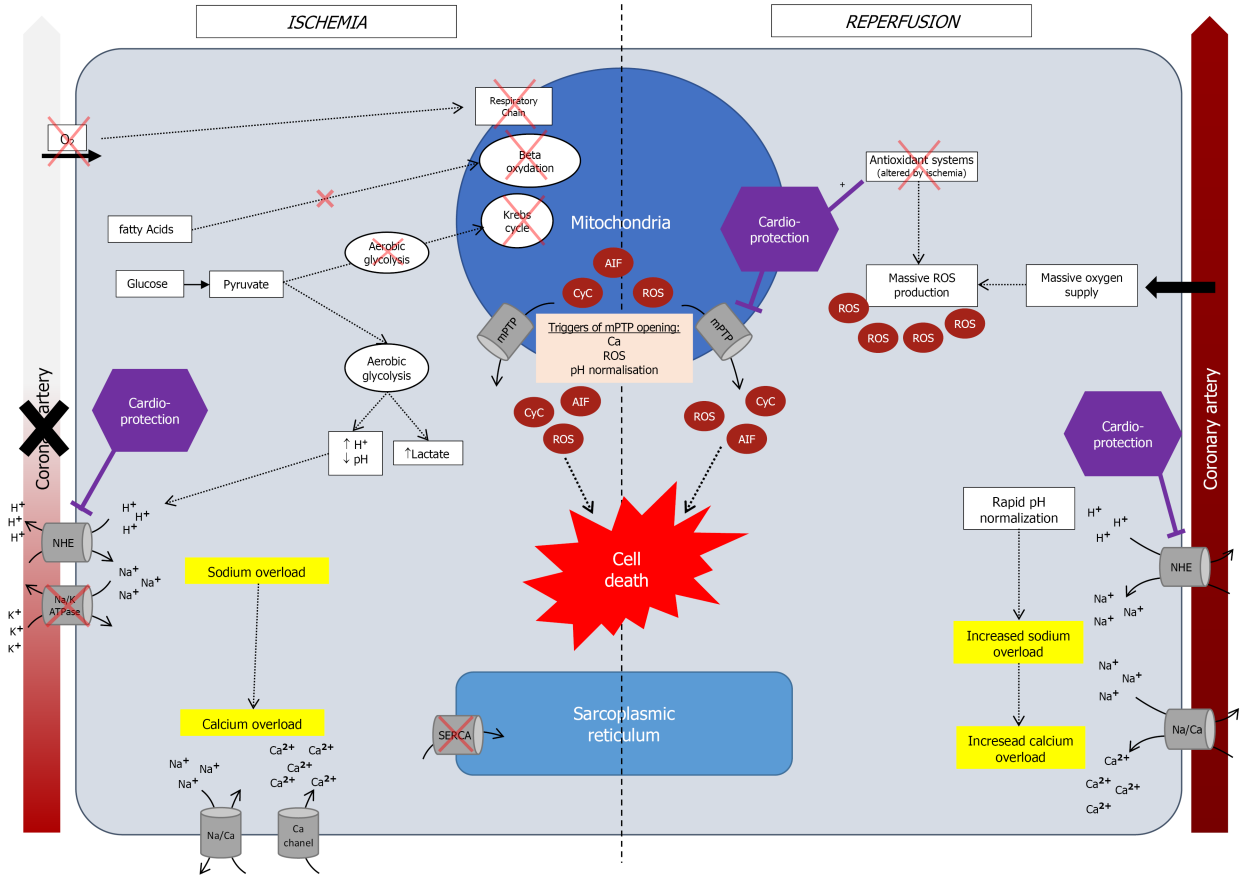

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Figure 1 Simplified physiopathology of ischemia/reperfusion injuries and cardio-protection. During ischemia, lack of oxygen leads to mitochondrial respiratory chain failure. ATP is produced mainly by using anaerobic glycolysis leading to acidosis and increase in lactate. Intracellular accumulation of H^+ activates the Na/H exchanger causing a sodium overload and calcium overload. All of these phenomenon result in mitochondrial permeability transition pore (mPTP) opening and release of reactive oxygen species (ROS), cytochrome C and AIF leading to cell death. During reperfusion, sudden oxygen supply led to massive ROS formation that are not eliminated by antioxidant systems (which have been damaged by ischemia). The rapid pH normalization increased the sodium and calcium overload (= pH paradox). mPTP opening is also increased. Cardio-protection strategies lead to inhibition of NHE, mPTP opening, or restauration of antioxidant systems. AIF: Apoptosis inducing factor; CyC: Cytochrome C; mPTP: Mitochondrial permeability transition pore; NHE: Na/H exchanger; ROS: Reactive oxygen species.

more lasting effects have been recently highlighted suggesting the role of protein synthesis [inducible nitric oxide (NO) synthase, cyclooxygenase, aldose reductase, superoxide dismutase][18]. Elements which are thought to mediate preconditioning benefit include but are not limited to adenosine, bradykinin or mechanical stretch activating various intracellular signaling pathways including RISK-pathway (increasing AKT and ERK1/2) and SAFE-pathway (increasing JAK and STAT) whose end targets are inhibition of mPTP opening, inhibition of Na/H exchanger or upregulation of antioxidant systems (superoxide dismutase, aldose reductase, *etc.*)[18,38].

Although promising, preconditioning is not reliable in clinical practice since it could not be used before acute coronary syndrome because of the brief effects of such procedure or the unpredictability of ACS. Hence, preconditioning could only be used in patient before CABG, by cross-clamping the aorta and then releasing for several minutes. Studies showed that it decreased post-operative ventricular arrhythmias, inotrope use and limited ICU stay[39].

On the other end, ischemic postconditioning consists in the same procedure, performed after the ischemic event, during reperfusion procedures. Similarly, it was associated with smaller infarct size[40, 41], a more progressive pH restoration, decreasing ROS production and calcium-induced mPTP opening, resulting in anti-apoptotic, anti-autophagic et anti-arrhythmic benefit[25].

While pre- and postconditioning aim at stimulating local anti-inflammatory pathways, remote conditioning consists in applying cycles of brief occlusion in other territories than that which is affected by ischemia (*i.e.*, neighboring coronary artery, limb). Theoretical advantages of this method lie in the fact that it may be applied at any time, is non-invasive and easily feasible. On top of the abovementioned mechanisms, additional systemic signal pathways may be involved with neuronal (peripheral sensory nerves, spinal cord, brainstems and vagal nerves) and humoral inducing a renal production of adenosine[42]. While this approach also aims at diminishing infarct size, mortality, and hospitalization for heart failure, phase III clinical trials failed to yield significant benefit, excepts in the most severe patients (cardiogenic shock or cardiac arrest)[18].

Yet, while multiple drugs have been tested, none showed clinical significance in human patients. Na/H exchangers inhibitors showed improvement in cardiovascular outcomes but increased stroke incidence[25,43,44]. Cyclosporine A, a nonspecific inhibitor of mPTP[45], promising initial results infarct, which were not translated in clinical studies[18]. Adenosine, acting as a vasodilator, was associated with pre- and postconditioning-like effects[46], through inhibition of mPTP opening[47]. Similarly, results were not conclusive in clinical trials[48]. Finally, NO was associated with potentially benefit in ischemia-reperfusion injuries by acting on oxygen consumption[49], platelet aggregation[50], leucocyte adhesion[51], and free radical scavenging[52].

These discrepancies between theoretical promises and disappointing clinical results require further research in the field, investigating novel pathways.

THE SGLT2 PATHWAY

Metabolic shift to a sparing substrate

In normal oxygenation conditions, myocardial mitochondrial oxidative metabolism exploits fatty acids (60%), glucose (30%), lactate and to a lesser degree ketones and amino acids, with a capacity to rapidly change substrates depending on workload or conditions. Under hypoxic conditions, myocardial substrate oxidation switches from free fatty acids to glucose and carbohydrate oxidation, because transformation of glucose to lactate is independent of oxygen supply[53]. During prolonged anaerobia, ketone becomes predominant as a resource. For instance, in animal models increasing the uptakes of 3-hydroxybutyrate (3HB) is associated with an improvement in cardiac function, pathologic cardiac remodeling, and oxygen consumption, whereas the capacity to oxidate substrate such as fatty acid is reduced[54]. Of note, 3HB is generated in the liver and may be used as a substrate for generating acetyl-CoA leading to increased production of NADH to drive energy transfer and ATP production.

Remarkably, in patient treated by SGLT2i, an uprising of ketone circulation was observed[55,56].

One of the hypotheses is that SGLT2i improves myocardial fuel metabolism, contractility, and cardiac efficiency by shifting catabolism away from lipids and glucose to that of ketone bodies[57]. Improved oxygen consumption and work efficiency at a mitochondrial level have been hypothesized[58]. Similarly as fasting, with the expected glucose depletion under SGLT2i, insulin-glucagon ratio is modulated, delivery of free fatty acids is increased to the liver which then stimulates ketogenesis[59]. Metabolomic profiles of patients with type 2 diabetes further support this hypothesis[55]. In addition to an expected reduction in glucose, SGLT2i increased 3HB levels suggesting an accrued utilization of ketone bodies. Moreover, increased intermediate metabolites of the urea cycle may indicate its use as well as amino-acids[55]. Remarkably, the same metabolic changes were observed in non-diabetic patients: Ferrannini *et al*[54] showed that SGLT2i reduced end-tissular glucose catabolism, accelerated lipolysis and fat oxidation. While these changes were more prominent after long-term exposition, an effect was observed as early as the first administration[53]. When compared to serum profiles of patients under corticoids treatment (widely tested in ischemia-injury model), SGLT2 might represent a different therapeutic candidate because of alternative energy income pathways involved[60]. A comparison between the metabolomic changes due to SGLT2i molecules as compared to glucocorticoids is available in Figure 2.

Because use of ketone bodies depends on the targeted organ, heart as well as kidneys may be those which benefit the most from an increase in 3HB[57]. Furthermore, similarly to an ischemia-hypoxia setting, during incremental atrial pacing, fractional extraction of 3HB persist, with improved energy efficiency; and a lower use of free fatty acids in low oxygenation conditions prevents the formation of ROS[59].

Of note, even if data from animal studies are promising and suggest benefit regarding infarct size and recovery, opposite signals appear when focusing on ketone bodies[58,61]. A recent work reported a suppression in ketone body utilization by myocardial during ischemia, based on levels of ß-hydroxybutyrate in patient presenting chest pain in a retrospective population[62]. Animal models with low-carbohydrate diet inducing mild nutritional ketosis showed a worse recovery and survival, more arrythmias after induced ischemia[63,64]. However, these contradictory results, well summarized in Kolwicz and al. review[65], only raise the need for additional studies at the metabolic level.

Inhibition of the NHE

SGLT2i were also associated with the inhibition of the NHE in myocardial cells[66]. We previously described the role of NHE in the homeostasis of ischemic cells, which induce oxidative stress with elevated cytosolic Na^+ and increased mitochondrial formation of ROS through a final intracellular calcium overload. The counterbalance of such mechanism requires the regeneration of antioxidative enzymes by mitochondria, relying on NADPH, indirectly produced by the Krebs cycle, in turn activated by intramitochondrial calcium[67]. NHE inhibitors were associated with cardioprotective features in animal models of acute myocardial infarction[68]. Moreover, a chronic inhibition of NHE was associated with improvement against cardiomyocyte injury, remodeling, and systolic dysfunction[69].

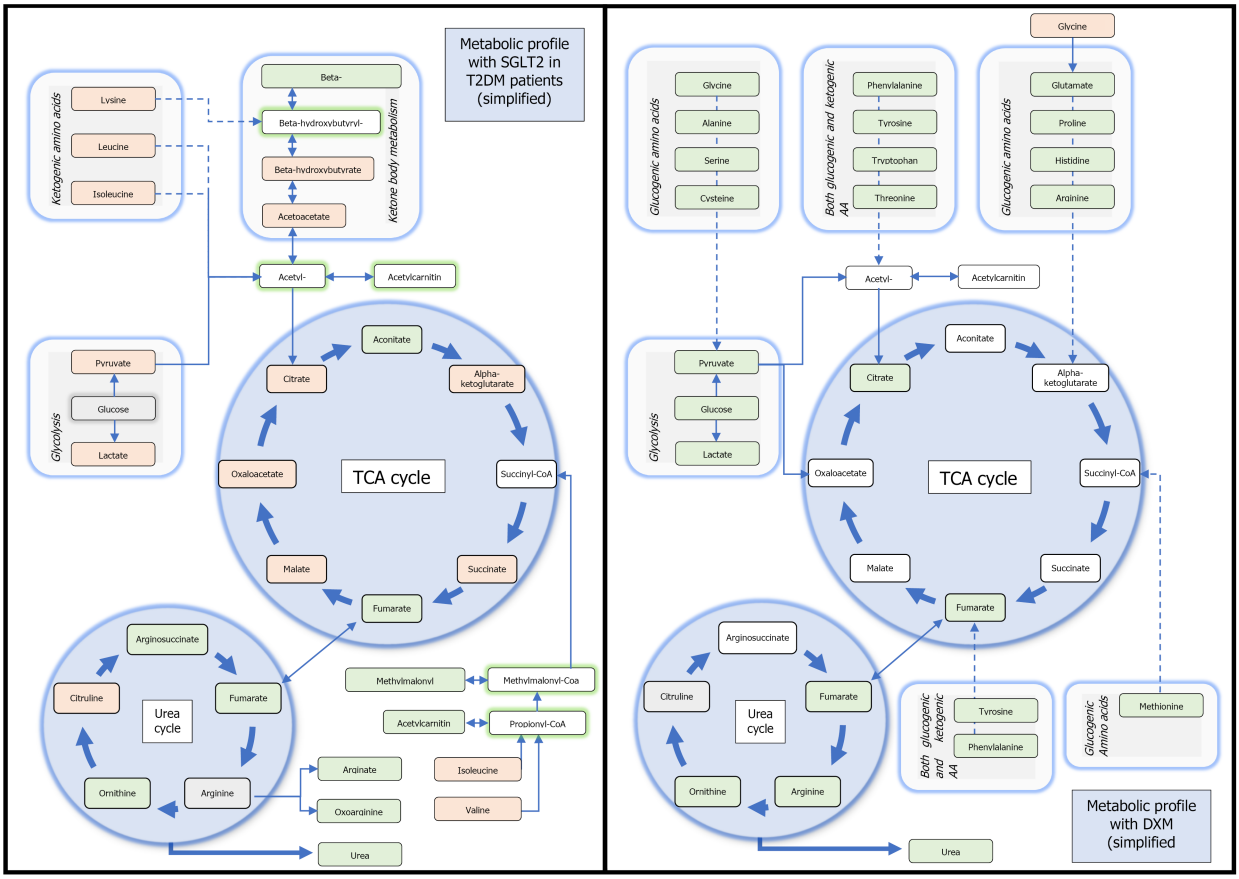

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Figure 2 Simplified comparison between metabolic profiles with sodium-glucose cotransporter-2 inhibitors or dexamethasone. Metabolites with observed high serum levels appear in light green, metabolites with supposed increased serum levels appear in highlight green with white center, those with decreased serum levels in gray center, those with unchanged serum levels appear in light orange and finally, those which remain untested appear in white. Incomes with sodium-glucose cotransporter-2 inhibitors suggest utilization of Ketone bodies and ketogenic amino acids as reactive for Krebs cycle, and indirectly urea cycle, when utilization of glucose is decreased. On the other hand, administration of dexamethasone is associated with elevated rates of glucogenic amino acids or ketogenic-glucogenic amino acids, concurring to Krebs cycle and urea cycle activations. TCA: Tricarboxylic acid cycle; DXM: Dexamethasone.

Remarkably, SGLT2i indirectly interacts with NHE. In mice, empagliflozin reversed the effects of ouabain (an agent increasing intracellular sodium)[70]. Moreover, this effect was independent from SGLT2 and indirectly caused a decreased activation of the Na^+/Ca^{2+} exchanger. The same results were observed with other SGLT2i (dapagliflozin, canagliflozin)[66].

This inhibition with empagliflozin was associated with lower rates of tumor necrosis factor alpha (TNF-α), attesting of a cell preservation and lowered inflammation through NHE inhibition.

Additional mechanisms which were hypothesized include: improved AMPK activation in myocytes [71], and cardio-fibroblasts[72]. In contrast, another study showed that concrete benefit on AMPK-pathway with SGLT2 in human cells and mouse cells *in vitro* seems unlikely because activation appeared with concentrations corresponding to the peak plasma concentrations of therapeutic doses [73].

In human cells, NHE inhibition showed similar results in atrial and ventricular myocytes, as compared to that of mice ventricular myocytes. Heart failure and atrial fibrillation were associated with increased NHE expression[74]. Finally, in human coronary endothelial cells, empagliflozin was associated with a similar reduction of oxidative stress supporting the previous hypothesis[75].

Positive effects of inhibition of NHE are not limited to better myocardial function, ionic homeostasis, or reduction of myocyte ischemic inflammation. Empagliflozin and canagliflozin in short-term treatment enhanced coronary vasodilation through NHE inhibition[66], whereas dapagliflozin needed a more prolonged treatment to reach comparable effect[76]. However, in cases of acute inflammation, a non-specific vasodilatation may occur, making it difficult to interpret supposed effect of inhibition of SGLT2[77].

Prevention of arrythmia and sudden death in ischemia-reperfusion injury

Sudden deaths and ventricular arrythmias may occur after acute ischemia and reperfusion events, and SGLT2i were associated with fewer such events. Yet, because SGLT2i do not inherently feature anti-arrhythmic properties, several mechanisms have been hypothesized[78]. An improved ionic homeo-

stasis through NHE inhibition has been suggested in the DAPA-HF trial, where 5.9% of the subjects assigned to the dapagliflozin group experimented serious rhythmic event (sudden death, cardiac arrest, ventricular arrythmias), with 7.4% in the placebo group[11]. In animal models, pre-treatment with empagliflozin reduced the incidence of reperfusion-induced ventricular arrythmia after an ischemia/reperfusion event, with the participation of the ERK1/2 pathway, involved in the RISK reperfusion-signaling pathway[79].

Role of the autonomous nervous system has also been investigated. In 2020, effects of empagliflozin *vs* placebo on cardiac sympathetic activity in acute myocardial infarction patients with T2DM (EMBODY Trial) compared empagliflozin with placebo for various electrocardiographic parameters. Heart rate variability, heart rate turbulence and electrocardiographic variations were recorded after acute myocardial infarction. Authors aimed to assess the variables associated with lethal ventricular arrhythmias. With a 6-mo-follow-up, a difference was observed between the two groups regarding sympathetic and parasympathetic stimulation[80]. Of note, to date, no study described these elements in the first few hours after an ischemic event index.

Finally, in a recent meta-analysis which analyzed the effects of SGLT2i on atrial arrythmia, sudden death and ventricular arrythmia which included 34 trials in patients with diabetes, use of SGLT2i were protective towards atrial arrythmia and sudden cardiac death, albeit several limitations existed[81].

Even if ionic homeostasis is the main hypothesis for the observed data, a plausible mechanism concurring to these results may lie on inhibition of platelet function, and antithrombin generation observed with SGLT2i. Unbalanced platelet activation and coagulation disturbance have been described during ischemic stress and associated with arrhythmia. SGLT2i have recently been associated with antiplatelet and antithrombotic features. Empagliflozin and dapagliflozin partially reduced the effects of stearic acid, an inflammatory agent inducing oxidative stress and impaired endothelial repair processes. As a result, platelets were less activated, in addition to that of ADP inhibition[82]. In male mice with T2DM model, administration of dapagliflozin showed a decreased activation and recruitment with an improved thrombin-platelet-mediated inflammation profile *in vivo* and less activated platelet with thrombin stimulation or CRP. Prolonged treatment did not affect hemostasis suggesting safety of utilization[83]. Gliflozin *via* NHE inhibition participate to maintain endothelial function[84] and endothelial production of NO. In a recent study, pharmacological analysis *in vitro* suggested that the gliflozin's antiplatelet activity synergize with NO and prostacyclin[85]. Substantial evidence sustaining an intricated mechanism.

Taken altogether, these elements encourage to explore concrete platelet and hemostasis parameters with SGLT2i in ischemic situation, to sustain a potential benefit in ischemic-reperfusion context.

EXPERIMENTAL MODELS

Models of myocardial ischemia-reperfusion

Beyond the theoretical data and focused exploratory clinical investigations, many animal models have been developed to assess the benefits of SGLT2 inhibitors in ischemia-reperfusion.

Acute administration of canagliflozin in male rat models of myocardial infarction showed decrease in infarct size, improved left ventricular systolic and diastolic function during and after ischemia, and decreased ROS[86]. Similar results were obtained with dapagliflozin[61], and the delay before the first ventricular arrythmia was lower when treated by SGLT2i. An improved communication between cardiac cells with preserved phosphorylation of gap junction protein connexin-43 was suggested[87,88]. Empagliflozin also showed similar results: reduced infarction size, better ventricular parameters, reduced systemic inflammation and ROS production, in acute or chronic administration[89,90]. The role of STAT3 phosphorylation was observed in several models[89-91]. Even if the beneficial mechanism is not yet fully determined, acute lowering of the blood glucose might be one of the potential hypothesis [92]. Interestingly, dipeptidyl peptidase 4 inhibitors were also compared to SGLT2i in murine models: SGLT2i showed greater efficacy than dipeptidyl peptidase 4 inhibitors to improve metabolic impairments and left ventricular function[93].

Recently, 16 independent animal models experiments which compared SGLT2i to control, and included 224 subjects overall, were summarized in a recent meta-analysis[94]. Regardless of diabetes, SGLT2is were significantly associated with fewer myocardial ischemia-reperfusion injuries and infarct size. Additionally, systemic treatment performed better than local administration, and longer-term treatment was associated with better results.

Other organ models

On top of myocardial protection, other organs have been tested.

In a model of lung injury due to ischemia-reperfusion, empagliflozin was tested on respiratory function, tissular and cellular analyses. Similarly, as in cardiac usage, SGLT2i was associated with lower levels of circulating cytokines in bronchoalveolar liquids, those were dependent on improved phosphorylation of pulmonary ERK1/2[95].

In models of ischemia-reperfusion-induced kidney injury, dapagliflozin was associated decreased biomarkers of renal failure (blood urea and creatinine) and fewer tubular injuries. Furthermore, under hypoxic condition, dapagliflozin reversed cellular death. Similarly, as in heart and lung, phosphorylation of AMPK and ERK1/2 was improved[96]. Remarkably, similar observations were made in non-diabetic rats[97].

Finally, in neurons, SGLT2i may interact with SGLT2 and SGLT1, expressed in human center nervous system[98]. Similar ischemia-reperfusion injuries may be performed in neurons, and empagliflozin in was associated with smaller infarct size and improved neuronal functions than in control rats. The main pathway studied was the HIF-1α/VEGF cascade, on which suppression of neuronal expression of Caspase-3 by empagliflozin had positive neuronal effects[99]. Moreover, role of Caspase-3 repression in hyperglycemic rats suggested an association between empagliflozin use and a decrease in TNF-α[100].

CONCLUSION

Beyond the cardiovascular benefits observed in patients with chronic heart failure treated by SGLT2i, data from large clinical trials including EMPA-REG or DAPA-HF may suggest a benefit through ischemia-reperfusion events. The inhibition of the NHE may play a pivotal role in such cardioprotective feature and further investigations towards the immunomodulatory properties of SGLT2i drug-class are warranted.

FOOTNOTES

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REFERENCES

1 **Zinman B**, Lachin JM, Inzucchi SE. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2016; **374**: 1094 [PMID: 26981940 DOI: 10.1056/NEJMc1600827]
2 **McMurray JJV**, Docherty KF, Jhund PS. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. Reply. *N Engl J Med* 2020; **382**: 973 [PMID: 32130823 DOI: 10.1056/NEJMc1917241]
3 **McDonagh TA**, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, Burri H, Butler J, Čelutkienė J, Chioncel O, Cleland JGF, Coats AJS, Crespo-Leiro MG, Farmakis D, Gilard M, Heymans S, Hoes AW, Jaarsma T, Jankowska EA, Lainscak M, Lam CSP, Lyon AR, McMurray JJV, Mebazaa A, Mindham R, Muneretto C, Francesco Piepoli M, Price S, Rosano GMC, Ruschitzka F, Kathrine Skibelund A; ESC Scientific Document Group. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). With the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2022; **24**: 4-131 [PMID: 35083827 DOI: 10.1002/ejhf.2333]
4 **Zinman B**, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 2117-2128 [PMID: 26378978 DOI: 10.1056/NEJMoa1504720]
5 **Fitchett D**, Inzucchi SE, Cannon CP, McGuire DK, Scirica BM, Johansen OE, Sambevski S, Kaspers S, Pfarr E, George JT, Zinman B. Empagliflozin Reduced Mortality and Hospitalization for Heart Failure Across the Spectrum of Cardiovascular Risk in the EMPA-REG OUTCOME Trial. *Circulation* 2019; **139**: 1384-1395 [PMID: 30586757 DOI: 10.1161/CIRCULATIONAHA.118.037778]
6 **Verma S**, Mazer CD, Fitchett D, Inzucchi SE, Pfarr E, George JT, Zinman B. Empagliflozin reduces cardiovascular

events, mortality and renal events in participants with type 2 diabetes after coronary artery bypass graft surgery: subanalysis of the EMPA-REG OUTCOME® randomised trial. *Diabetologia* 2018; **61**: 1712-1723 [PMID: 29777264 DOI: 10.1007/s00125-018-4644-9]

7 Correction to: Effect of Empagliflozin on the Clinical Stability of Patients With Heart Failure and a Reduced Ejection Fraction: The EMPEROR-Reduced Trial. *Circulation* 2021; **143**: e30 [PMID: 33493036 DOI: 10.1161/CIR.0000000000000954]

8 **Anker SD**, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, Brunner-La Rocca HP, Choi DJ, Chopra V, Chuquiure-Valenzuela E, Giannetti N, Gomez-Mesa JE, Janssens S, Januzzi JL, Gonzalez-Juanatey JR, Merkely B, Nicholls SJ, Perrone SV, Piña IL, Ponikowski P, Senni M, Sim D, Spinar J, Squire I, Taddei S, Tsutsui H, Verma S, Vinereanu D, Zhang J, Carson P, Lam CSP, Marx N, Zeller C, Sattar N, Jamal W, Schnaidt S, Schnee JM, Brueckmann M, Pocock SJ, Zannad F, Packer M; EMPEROR-Preserved Trial Investigators. Empagliflozin in Heart Failure with a Preserved Ejection Fraction. *N Engl J Med* 2021; **385**: 1451-1461 [PMID: 34449189 DOI: 10.1056/NEJMoa2107038]

9 **Nassif ME**, Windsor SL, Borlaug BA, Kitzman DW, Shah SJ, Tang F, Khariton Y, Malik AO, Khumri T, Umpierrez G, Lamba S, Sharma K, Khan SS, Chandra L, Gordon RA, Ryan JJ, Chaudhry SP, Joseph SM, Chow CH, Kanwar MK, Pursley M, Siraj ES, Lewis GD, Clemson BS, Fong M, Kosiborod MN. The SGLT2 inhibitor dapagliflozin in heart failure with preserved ejection fraction: a multicenter randomized trial. *Nat Med* 2021; **27**: 1954-1960 [PMID: 34711976 DOI: 10.1038/s41591-021-01536-x]

10 **Bell RM**, Yellon DM. SGLT2 inhibitors: hypotheses on the mechanism of cardiovascular protection. *Lancet Diabetes Endocrinol* 2018; **6**: 435-437 [PMID: 29030201 DOI: 10.1016/S2213-8587(17)30314-5]

11 **Curtain JP**, Docherty KF, Jhund PS, Petrie MC, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Bengtsson O, Langkilde AM, Sjöstrand M, Solomon SD, McMurray JJV. Effect of dapagliflozin on ventricular arrhythmias, resuscitated cardiac arrest, or sudden death in DAPA-HF. *Eur Heart J* 2021; **42**: 3727-3738 [PMID: 34448003 DOI: 10.1093/eurheartj/ehab560]

12 **Ibanez B**, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimský P; ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2018; **39**: 119-177 [PMID: 28886621 DOI: 10.1093/eurheartj/ehx393]

13 **Park J**, Choi KH, Lee JM, Kim HK, Hwang D, Rhee TM, Kim J, Park TK, Yang JH, Song YB, Choi JH, Hahn JY, Choi SH, Koo BK, Chae SC, Cho MC, Kim CJ, Kim JH, Jeong MH, Gwon HC, Kim HS; KAMIR-NIH (Korea Acute Myocardial Infarction Registry–National Institutes of Health) Investigators. Prognostic Implications of Door-to-Balloon Time and Onset-to-Door Time on Mortality in Patients With ST -Segment-Elevation Myocardial Infarction Treated With Primary Percutaneous Coronary Intervention. *J Am Heart Assoc* 2019; **8**: e012188 [PMID: 31041869 DOI: 10.1161/JAHA.119.012188]

14 **Heusch G**. Myocardial Ischemia: Lack of Coronary Blood Flow or Myocardial Oxygen Supply/Demand Imbalance? *Circ Res* 2016; **119**: 194-196 [PMID: 27390331 DOI: 10.1161/CIRCRESAHA.116.308925]

15 **Maroko PR**, Libby P, Ginks WR, Bloor CM, Shell WE, Sobel BE, Ross J Jr. Coronary artery reperfusion. I. Early effects on local myocardial function and the extent of myocardial necrosis. *J Clin Invest* 1972; **51**: 2710-2716 [PMID: 5056663 DOI: 10.1172/jci107090]

16 **Ibáñez B**, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. *J Am Coll Cardiol* 2015; **65**: 1454-1471 [PMID: 25857912 DOI: 10.1016/j.jacc.2015.02.032]

17 **Braunwald E**, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Invest* 1985; **76**: 1713-1719 [PMID: 4056048 DOI: 10.1172/jci112160]

18 **Heusch G**. Myocardial ischaemia-reperfusion injury and cardioprotection in perspective. *Nat Rev Cardiol* 2020; **17**: 773-789 [PMID: 32620851 DOI: 10.1038/s41569-020-0403-y]

19 **Stone GW**, Selker HP, Thiele H, Patel MR, Udelson JE, Ohman EM, Maehara A, Eitel I, Granger CB, Jenkins PL, Nichols M, Ben-Yehuda O. Relationship Between Infarct Size and Outcomes Following Primary PCI: Patient-Level Analysis From 10 Randomized Trials. *J Am Coll Cardiol* 2016; **67**: 1674-1683 [PMID: 27056772 DOI: 10.1016/j.jacc.2016.01.069]

20 **Weman SM**, Karhunen PJ, Penttilä A, Järvinen AA, Salminen US. Reperfusion injury associated with one-fourth of deaths after coronary artery bypass grafting. *Ann Thorac Surg* 2000; **70**: 807-812 [PMID: 11016314 DOI: 10.1016/s0003-4975(00)01638-6]

21 **Costa MA**, Carere RG, Lichtenstein SV, Foley DP, de Valk V, Lindenboom W, Roose PC, van Geldorp TR, Macaya C, Castanon JL, Fernandez-Avilèz F, Gonzáles JH, Heyer G, Unger F, Serruys PW. Incidence, predictors, and significance of abnormal cardiac enzyme rise in patients treated with bypass surgery in the arterial revascularization therapies study (ARTS). *Circulation* 2001; **104**: 2689-2693 [PMID: 11723020 DOI: 10.1161/hc4701.099789]

22 **Turer AT**, Hill JA. Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy. *Am J Cardiol* 2010; **106**: 360-368 [PMID: 20643246 DOI: 10.1016/j.amjcard.2010.03.032]

23 **Heusch G**. Cardioprotection: chances and challenges of its translation to the clinic. *Lancet* 2013; **381**: 166-175 [PMID: 23095318 DOI: 10.1016/S0140-6736(12)60916-7]

24 **Jennings RB**, Reimer KA. Factors involved in salvaging ischemic myocardium: effect of reperfusion of arterial blood. *Circulation* 1983; **68**: I25-I36 [PMID: 6861325]

25 **Benhabbouche S**, Crola da Silva C, Abrial M, Ferrera R. [The basis of ischemia-reperfusion and myocardial protection]. *Ann Fr Anesth Reanim* 2011; **30** Suppl 1: S2-16 [PMID: 21703480 DOI: 10.1016/S0750-7658(11)70002-8]

26 **Jennings RB**, Murry CE, Steenbergen C Jr, Reimer KA. Development of cell injury in sustained acute ischemia. *Circulation* 1990; **82**: II2-I12 [PMID: 2394018]

27 **Chassot PG**. Precis d'anesthesie cardiaque 5. [cited 1 April 2022]. Available from: https://www.pac5.ch/fr/node/1076/take

28 **Herzig JW**, Peterson JW, Solaro RJ, Rüegg JC. Phosphate and vanadate reduce the efficiency of the chemo-mechanical energy transformation in cardiac muscle. *Adv Exp Med Biol* 1982; **151**: 267-281 [PMID: 6217726 DOI: 10.1007/978-1-4684-4259-5_33]

29 **Berdeaux A**, Tissier R, Couvreur N, Salouage I, Ghaleb B. [Heart rate reduction: beneficial effects in heart failure and post-infarcted myocardium]. *Therapie* 2009; **64**: 87-91 [PMID: 19664401 DOI: 10.2515/therapie/2009013]

30 **Tani M**, Neely JR. Role of intracellular Na+ in Ca2+ overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. Possible involvement of H+-Na+ and Na+-Ca2+ exchange. *Circ Res* 1989; **65**: 1045-1056 [PMID: 2551525 DOI: 10.1161/01.res.65.4.1045]

31 **Schild L**, Reiser G. Oxidative stress is involved in the permeabilization of the inner membrane of brain mitochondria exposed to hypoxia/reoxygenation and low micromolar Ca2+. *FEBS J* 2005; **272**: 3593-3601 [PMID: 16008559 DOI: 10.1111/j.1742-4658.2005.04781.x]

32 **Zorov DB**, Juhaszova M, Yaniv Y, Nuss HB, Wang S, Sollott SJ. Regulation and pharmacology of the mitochondrial permeability transition pore. *Cardiovasc Res* 2009; **83**: 213-225 [PMID: 19447775 DOI: 10.1093/cvr/cvp151]

33 **Kuppusamy P**, Zweier JL. Characterization of free radical generation by xanthine oxidase. Evidence for hydroxyl radical generation. *J Biol Chem* 1989; **264**: 9880-9884 [PMID: 2542334]

34 **Thompson-Gorman SL**, Zweier JL. Evaluation of the role of xanthine oxidase in myocardial reperfusion injury. *J Biol Chem* 1990; **265**: 6656-6663 [PMID: 2157706]

35 **Verma S**, Fedak PW, Weisel RD, Butany J, Rao V, Maitland A, Li RK, Dhillon B, Yau TM. Fundamentals of reperfusion injury for the clinical cardiologist. *Circulation* 2002; **105**: 2332-2336 [PMID: 12021216 DOI: 10.1161/01.cir.0000016602.96363.36]

36 **Lemasters JJ**, Bond JM, Chacon E, Harper IS, Kaplan SH, Ohata H, Trollinger DR, Herman B, Cascio WE. The pH paradox in ischemia-reperfusion injury to cardiac myocytes. *EXS* 1996; **76**: 99-114 [PMID: 8805791 DOI: 10.1007/978-3-0348-8988-9_7]

37 **Bond JM**, Herman B, Lemasters JJ. Protection by acidotic pH against anoxia/reoxygenation injury to rat neonatal cardiac myocytes. *Biochem Biophys Res Commun* 1991; **179**: 798-803 [PMID: 1898402 DOI: 10.1016/0006-291x(91)91887-i]

38 **Murry CE**, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; **74**: 1124-1136 [PMID: 3769170 DOI: 10.1161/01.cir.74.5.1124]

39 **Walsh SR**, Tang TY, Kullar P, Jenkins DP, Dutka DP, Gaunt ME. Ischaemic preconditioning during cardiac surgery: systematic review and meta-analysis of perioperative outcomes in randomised clinical trials. *Eur J Cardiothorac Surg* 2008; **34**: 985-994 [PMID: 18783958 DOI: 10.1016/j.ejcts.2008.07.062]

40 **Zhao ZQ**, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003; **285**: H579-H588 [PMID: 12860564 DOI: 10.1152/ajpheart.01064.2002]

41 **Lie RH**, Hasenkam JM, Nielsen TT, Poulsen R, Sloth E. Post-conditioning reduces infarct size in an open-chest porcine acute ischemia-reperfusion model. *Acta Anaesthesiol Scand* 2008; **52**: 1188-1193 [PMID: 18823456 DOI: 10.1111/j.1399-6576.2008.01756.x]

42 **Kerendi F**, Kin H, Halkos ME, Jiang R, Zatta AJ, Zhao ZQ, Guyton RA, Vinten-Johansen J. Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size *via* endogenous activation of adenosine receptors. *Basic Res Cardiol* 2005; **100**: 404-412 [PMID: 15965583 DOI: 10.1007/s00395-005-0539-2]

43 **Chakrabarti S**, Hoque AN, Karmazyn M. A rapid ischemia-induced apoptosis in isolated rat hearts and its attenuation by the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). *J Mol Cell Cardiol* 1997; **29**: 3169-3174 [PMID: 9405190 DOI: 10.1006/jmcc.1997.0561]

44 **Mentzer RM Jr**, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B, Haverich A, Knight J, Menasché P, Myers ML, Nicolau J, Simoons M, Thulin L, Weisel RD; EXPEDITION Study Investigators. Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg* 2008; **85**: 1261-1270 [PMID: 18355507 DOI: 10.1016/j.athoracsur.2007.10.054]

45 **Piot C**, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, Elbelghiti R, Cung TT, Bonnefoy E, Angoulvant D, Macia C, Raczka F, Sportouch C, Gahide G, Finet G, André-Fouët X, Revel D, Kirkorian G, Monassier JP, Derumeaux G, Ovize M. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 2008; **359**: 473-481 [PMID: 18669426 DOI: 10.1056/NEJMoa071142]

46 **Liu GS**, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. *Circulation* 1991; **84**: 350-356 [PMID: 2060105 DOI: 10.1161/01.cir.84.1.350]

47 **Xi J**, McIntosh R, Shen X, Lee S, Chanoit G, Criswell H, Zvara DA, Xu Z. Adenosine A2A and A2B receptors work in concert to induce a strong protection against reperfusion injury in rat hearts. *J Mol Cell Cardiol* 2009; **47**: 684-690 [PMID: 19695259 DOI: 10.1016/j.yjmcc.2009.08.009]

48 **Mahaffey KW**, Puma JA, Barbagelata NA, DiCarli MF, Leesar MA, Browne KF, Eisenberg PR, Bolli R, Casas AC, Molina-Viamonte V, Orlandi C, Blevins R, Gibbons RJ, Califf RM, Granger CB. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: results of a multicenter, randomized, placebo-controlled trial: the Acute Myocardial Infarction STudy of ADenosine (AMISTAD) trial. *J Am Coll Cardiol* 1999; **34**: 1711-1720 [PMID: 10577561 DOI: 10.1016/s0735-1097(99)00418-0]

49 **Loke KE**, McConnell PI, Tuzman JM, Shesely EG, Smith CJ, Stackpole CJ, Thompson CI, Kaley G, Wolin MS, Hintze TH. Endogenous endothelial nitric oxide synthase-derived nitric oxide is a physiological regulator of myocardial oxygen consumption. *Circ Res* 1999; **84**: 840-845 [PMID: 10205152 DOI: 10.1161/01.res.84.7.840]

50 **Radomski MW**, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* 1987; **2**: 1057-1058 [PMID: 2889967 DOI: 10.1016/s0140-6736(87)91481-4]

51 **Ma XL**, Weyrich AS, Lefer DJ, Lefer AM. Diminished basal nitric oxide release after myocardial ischemia and

reperfusion promotes neutrophil adherence to coronary endothelium. *Circ Res* 1993; **72**: 403-412 [PMID: 8418991 DOI: 10.1161/01.res.72.2.403]

52 **Beckman JS**, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1990; **87**: 1620-1624 [PMID: 2154753 DOI: 10.1073/pnas.87.4.1620]

53 **Ferrannini E**, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, Mari A, Pieber TR, Muscelli E. Shift to Fatty Substrate Utilization in Response to Sodium-Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes. *Diabetes* 2016; **65**: 1190-1195 [PMID: 26861783 DOI: 10.2337/db15-1356]

54 **Horton JL**, Davidson MT, Kurishima C, Vega RB, Powers JC, Matsuura TR, Petucci C, Lewandowski ED, Crawford PA, Muoio DM, Recchia FA, Kelly DP. The failing heart utilizes 3-hydroxybutyrate as a metabolic stress defense. *JCI Insight* 2019; **4** [PMID: 30668551 DOI: 10.1172/jci.insight.124079]

55 **Kappel BA**, Lehrke M, Schütt K, Artati A, Adamski J, Lebherz C, Marx N. Effect of Empagliflozin on the Metabolic Signature of Patients With Type 2 Diabetes Mellitus and Cardiovascular Disease. *Circulation* 2017; **136**: 969-972 [PMID: 28874423 DOI: 10.1161/CIRCULATIONAHA.117.029166]

56 **Bedi KC Jr**, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, Rame JE. Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. *Circulation* 2016; **133**: 706-716 [PMID: 26819374 DOI: 10.1161/CIRCULATIONAHA.115.017545]

57 **Mudaliar S**, Alloju S, Henry RR. Can a Shift in Fuel Energetics Explain the Beneficial Cardiorenal Outcomes in the EMPA-REG OUTCOME Study? *Diabetes Care* 2016; **39**: 1115-1122 [PMID: 27289124 DOI: 10.2337/dc16-0542]

58 **Yurista SR**, Silljé HHW, Oberdorf-Maass SU, Schouten EM, Pavez Giani MG, Hillebrands JL, van Goor H, van Veldhuisen DJ, de Boer RA, Westenbrink BD. Sodium-glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction. *Eur J Heart Fail* 2019; **21**: 862-873 [PMID: 31033127 DOI: 10.1002/ejhf.1473]

59 **Ferrannini E**, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis. *Diabetes Care* 2016; **39**: 1108-1114 [PMID: 27289126 DOI: 10.2337/dc16-0330]

60 **Bordag N**, Klie S, Jürchott K, Vierheller J, Schiewe H, Albrecht V, Tonn JC, Schwartz C, Schichor C, Selbig J. Glucocorticoid (dexamethasone)-induced metabolome changes in healthy males suggest prediction of response and side effects. *Sci Rep* 2015; **5**: 15954 [PMID: 26526738 DOI: 10.1038/srep15954]

61 **Lahnwong S**, Palee S, Apaijai N, Sriwichaiin S, Kerdphoo S, Jaiwongkam T, Chattipakorn SC, Chattipakorn N. Acute dapagliflozin administration exerts cardioprotective effects in rats with cardiac ischemia/reperfusion injury. *Cardiovasc Diabetol* 2020; **19**: 91 [PMID: 32539724 DOI: 10.1186/s12933-020-01066-9]

62 **Arima Y**, Izumiya Y, Ishida T, Takashio S, Ishii M, Sueta D, Fujisue K, Sakamoto K, Kaikita K, Tsujita K. Myocardial Ischemia Suppresses Ketone Body Utilization. *J Am Coll Cardiol* 2019; **73**: 246-247 [PMID: 30408507 DOI: 10.1016/j.jacc.2018.10.040]

63 **Liu J**, Wang P, Douglas SL, Tate JM, Sham S, Lloyd SG. Impact of high-fat, low-carbohydrate diet on myocardial substrate oxidation, insulin sensitivity, and cardiac function after ischemia-reperfusion. *Am J Physiol Heart Circ Physiol* 2016; **311**: H1-H10 [PMID: 27199129 DOI: 10.1152/ajpheart.00809.2015]

64 **Wang P**, Tate JM, Lloyd SG. Low carbohydrate diet decreases myocardial insulin signaling and increases susceptibility to myocardial ischemia. *Life Sci* 2008; **83**: 836-844 [PMID: 18951908 DOI: 10.1016/j.lfs.2008.09.024]

65 **Kolwicz SC Jr**. Ketone Body Metabolism in the Ischemic Heart. *Front Cardiovasc Med* 2021; **8**: 789458 [PMID: 34950719 DOI: 10.3389/fcvm.2021.789458]

66 **Uthman L**, Baartscheer A, Bleijlevens B, Schumacher CA, Fiolet JWT, Koeman A, Jancev M, Hollmann MW, Weber NC, Coronel R, Zuurbier CJ. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na^+/H^+ exchanger, lowering of cytosolic Na^+ and vasodilation. *Diabetologia* 2018; **61**: 722-726 [PMID: 29197997 DOI: 10.1007/s00125-017-4509-7]

67 **Kohlhaas M**, Liu T, Knopp A, Zeller T, Ong MF, Böhm M, O'Rourke B, Maack C. Elevated cytosolic Na+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation* 2010; **121**: 1606-1613 [PMID: 20351235 DOI: 10.1161/CIRCULATIONAHA.109.914911]

68 **Avkiran M**, Marber MS. Na(+)/H(+) exchange inhibitors for cardioprotective therapy: progress, problems and prospects. *J Am Coll Cardiol* 2002; **39**: 747-753 [PMID: 11869836 DOI: 10.1016/s0735-1097(02)01693-5]

69 **Jun S**, Aon MA, Paolocci N. Empagliflozin and HFrEF: Known and Possible Benefits of NHE1 Inhibition. *JACC Basic Transl Sci* 2019; **4**: 841-844 [PMID: 31999274 DOI: 10.1016/j.jacbts.2019.10.005]

70 **Peng X**, Li L, Lin R, Wang X, Liu X, Li Y, Ma C, Ruan Y, Liu N. Empagliflozin Ameliorates Ouabain-Induced Na^+ and Ca^{2+} Dysregulations in Ventricular Myocytes in an Na^+-Dependent Manner. *Cardiovasc Drugs Ther* 2022 [PMID: 34982348 DOI: 10.1007/s10557-021-07311-x]

71 **Li X**, Lu Q, Qiu Y, do Carmo JM, Wang Z, da Silva AA, Mouton A, Omoto ACM, Hall ME, Li J, Hall JE. Direct Cardiac Actions of the Sodium Glucose Co-Transporter 2 Inhibitor Empagliflozin Improve Myocardial Oxidative Phosphorylation and Attenuate Pressure-Overload Heart Failure. *J Am Heart Assoc* 2021; **10**: e018298 [PMID: 33719499 DOI: 10.1161/JAHA.120.018298]

72 **Ye Y**, Jia X, Bajaj M, Birnbaum Y. Dapagliflozin Attenuates Na^+/H^+ Exchanger-1 in Cardiofibroblasts *via* AMPK Activation. *Cardiovasc Drugs Ther* 2018; **32**: 553-558 [PMID: 30367338 DOI: 10.1007/s10557-018-6837-3]

73 **Hawley SA**, Ford RJ, Smith BK, Gowans GJ, Mancini SJ, Pitt RD, Day EA, Salt IP, Steinberg GR, Hardie DG. The Na+/Glucose Cotransporter Inhibitor Canagliflozin Activates AMPK by Inhibiting Mitochondrial Function and Increasing Cellular AMP Levels. *Diabetes* 2016; **65**: 2784-2794 [PMID: 27381369 DOI: 10.2337/db16-0058]

74 **Trum M**, Riechel J, Lebek S, Pabel S, Sossalla ST, Hirt S, Arzt M, Maier LS, Wagner S. Empagliflozin inhibits Na^+ /H^+ exchanger activity in human atrial cardiomyocytes. *ESC Heart Fail* 2020 [PMID: 32946200 DOI: 10.1002/ehf2.13024]

75 **Uthman L**, Li X, Baartscheer A, Schumacher CA, Baumgart P, Hermanides J, Preckel B, Hollmann MW, Coronel R, Zuurbier CJ, Weber NC. Empagliflozin reduces oxidative stress through inhibition of the novel inflammation/NHE/$[Na^+]_c$

/ROS-pathway in human endothelial cells. *Biomed Pharmacother* 2022; **146**: 112515 [PMID: 34896968 DOI: 10.1016/j.biopha.2021.112515]

76 **Cappetta D**, De Angelis A, Ciuffreda LP, Coppini R, Cozzolino A, Miccichè A, Dell'Aversana C, D'Amario D, Cianflone E, Scavone C, Santini L, Palandri C, Naviglio S, Crea F, Rota M, Altucci L, Rossi F, Capuano A, Urbanek K, Berrino L. Amelioration of diastolic dysfunction by dapagliflozin in a non-diabetic model involves coronary endothelium. *Pharmacol Res* 2020; **157**: 104781 [PMID: 32360273 DOI: 10.1016/j.phrs.2020.104781]

77 **Sherwood ER**, Toliver-Kinsky T. Mechanisms of the inflammatory response. *Best Pract Res Clin Anaesthesiol* 2004; **18**: 385-405 [PMID: 15212335 DOI: 10.1016/j.bpa.2003.12.002]

78 **Kolesnik E**, Scherr D, Rohrer U, Benedikt M, Manninger M, Sourij H, von Lewinski D. SGLT2 Inhibitors and Their Antiarrhythmic Properties. *Int J Mol Sci* 2022; **23** [PMID: 35163599 DOI: 10.3390/ijms23031678]

79 **Hu Z**, Ju F, Du L, Abbott GW. Empagliflozin protects the heart against ischemia/reperfusion-induced sudden cardiac death. *Cardiovasc Diabetol* 2021; **20**: 199 [PMID: 34607570 DOI: 10.1186/s12933-021-01392-6]

80 **Shimizu W**, Kubota Y, Hoshika Y, Mozawa K, Tara S, Tokita Y, Yodogawa K, Iwasaki YK, Yamamoto T, Takano H, Tsukada Y, Asai K, Miyamoto M, Miyauchi Y, Kodani E, Ishikawa M, Maruyama M, Ogano M, Tanabe J; EMBODY trial investigators. Effects of empagliflozin versus placebo on cardiac sympathetic activity in acute myocardial infarction patients with type 2 diabetes mellitus: the EMBODY trial. *Cardiovasc Diabetol* 2020; **19**: 148 [PMID: 32977831 DOI: 10.1186/s12933-020-01127-z]

81 **Fernandes GC**, Fernandes A, Cardoso R, Penalver J, Knijnik L, Mitrani RD, Myerburg RJ, Goldberger JJ. Association of SGLT2 inhibitors with arrhythmias and sudden cardiac death in patients with type 2 diabetes or heart failure: A meta-analysis of 34 randomized controlled trials. *Heart Rhythm* 2021; **18**: 1098-1105 [PMID: 33757845 DOI: 10.1016/j.hrthm.2021.03.028]

82 **Spigoni V**, Fantuzzi F, Carubbi C, Pozzi G, Masselli E, Gobbi G, Solini A, Bonadonna RC, Dei Cas A. Sodium-glucose cotransporter 2 inhibitors antagonize lipotoxicity in human myeloid angiogenic cells and ADP-dependent activation in human platelets: potential relevance to prevention of cardiovascular events. *Cardiovasc Diabetol* 2020; **19**: 46 [PMID: 32264868 DOI: 10.1186/s12933-020-01016-5]

83 **Kohlmorgen C**, Gerfer S, Feldmann K, Twarock S, Hartwig S, Lehr S, Klier M, Krüger I, Helten C, Keul P, Kahl S, Polzin A, Elvers M, Flögel U, Kelm M, Levkau B, Roden M, Fischer JW, Grandoch M. Dapagliflozin reduces thrombin generation and platelet activation: implications for cardiovascular risk reduction in type 2 diabetes mellitus. *Diabetologia* 2021; **64**: 1834-1849 [PMID: 34131781 DOI: 10.1007/s00125-021-05498-0]

84 **Park SH**, Belcastro E, Hasan H, Matsushita K, Marchandot B, Abbas M, Toti F, Auger C, Jesel L, Ohlmann P, Morel O, Schini-Kerth VB. Angiotensin II-induced upregulation of SGLT1 and 2 contributes to human microparticle-stimulated endothelial senescence and dysfunction: protective effect of gliflozins. *Cardiovasc Diabetol* 2021; **20**: 65 [PMID: 33726768 DOI: 10.1186/s12933-021-01252-3]

85 **Lescano CH**, Leonardi G, Torres PHP, Amaral TN, de Freitas Filho LH, Antunes E, Vicente CP, Anhê GF, Mónica FZ. The sodium-glucose cotransporter-2 (SGLT2) inhibitors synergize with nitric oxide and prostacyclin to reduce human platelet activation. *Biochem Pharmacol* 2020; **182**: 114276 [PMID: 33039417 DOI: 10.1016/j.bcp.2020.114276]

86 **Sayour AA**, Korkmaz-Icöz S, Loganathan S, Ruppert M, Sayour VN, Oláh A, Benke K, Brune M, Benkő R, Horváth EM, Karck M, Merkely B, Radovits T, Szabó G. Acute canagliflozin treatment protects against in vivo myocardial ischemia-reperfusion injury in non-diabetic male rats and enhances endothelium-dependent vasorelaxation. *J Transl Med* 2019; **17**: 127 [PMID: 30992077 DOI: 10.1186/s12967-019-1881-8]

87 **Lampe PD**, TenBroek EM, Burt JM, Kurata WE, Johnson RG, Lau AF. Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication. *J Cell Biol* 2000; **149**: 1503-1512 [PMID: 10871288 DOI: 10.1083/jcb.149.7.1503]

88 **Richards TS**, Dunn CA, Carter WG, Usui ML, Olerud JE, Lampe PD. Protein kinase C spatially and temporally regulates gap junctional communication during human wound repair *via* phosphorylation of connexin43 on serine368. *J Cell Biol* 2004; **167**: 555-562 [PMID: 15534005 DOI: 10.1083/jcb.200404142]

89 **Andreadou I**, Efentakis P, Balafas E, Togliatto G, Davos CH, Varela A, Dimitriou CA, Nikolaou PE, Maratou E, Lambadiari V, Ikonomidis I, Kostomitsopoulos N, Brizzi MF, Dimitriadis G, Iliodromitis EK. Empagliflozin Limits Myocardial Infarction *in Vivo* and Cell Death *in Vitro*: Role of STAT3, Mitochondria, and Redox Aspects. *Front Physiol* 2017; **8**: 1077 [PMID: 29311992 DOI: 10.3389/fphys.2017.01077]

90 **Nikolaou PE**, Efentakis P, Abu Qourah F, Femminò S, Makridakis M, Kanaki Z, Varela A, Tsoumani M, Davos CH, Dimitriou CA, Tasouli A, Dimitriadis G, Kostomitsopoulos N, Zuurbier CJ, Vlahou A, Klinakis A, Brizzi MF, Iliodromitis EK, Andreadou I. Chronic Empagliflozin Treatment Reduces Myocardial Infarct Size in Nondiabetic Mice Through STAT-3-Mediated Protection on Microvascular Endothelial Cells and Reduction of Oxidative Stress. *Antioxid Redox Signal* 2021; **34**: 551-571 [PMID: 32295413 DOI: 10.1089/ars.2019.7923]

91 **Lee TM**, Chang NC, Lin SZ. Dapagliflozin, a selective SGLT2 Inhibitor, attenuated cardiac fibrosis by regulating the macrophage polarization *via* STAT3 signaling in infarcted rat hearts. *Free Radic Biol Med* 2017; **104**: 298-310 [PMID: 28132924 DOI: 10.1016/j.freeradbiomed.2017.01.035]

92 **Zuurbier CJ**. Does acute treatment of dapagliflozin reduce cardiac infarct size through direct cardiac effects or reductions in blood glucose levels? *Cardiovasc Diabetol* 2020; **19**: 141 [PMID: 32950054 DOI: 10.1186/s12933-020-01119-z]

93 **Tanajak P**, Sa-Nguanmoo P, Sivasinprasasn S, Thummasorn S, Siri-Angkul N, Chattipakorn SC, Chattipakorn N. Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury. *J Endocrinol* 2018; **236**: 69-84 [PMID: 29142025 DOI: 10.1530/JOE-17-0457]

94 **Sayour AA**, Celeng C, Oláh A, Ruppert M, Merkely B, Radovits T. Sodium-glucose cotransporter 2 inhibitors reduce myocardial infarct size in preclinical animal models of myocardial ischaemia-reperfusion injury: a meta-analysis. *Diabetologia* 2021; **64**: 737-748 [PMID: 33483761 DOI: 10.1007/s00125-020-05359-2]

95 **Huang D**, Ju F, Du L, Liu T, Zuo Y, Abbott GW, Hu Z. Empagliflozin Protects against Pulmonary Ischemia/Reperfusion Injury *via* an Extracellular Signal-Regulated Kinases 1 and 2-Dependent Mechanism. *J Pharmacol Exp Ther* 2022; **380**: 230-241 [PMID: 34893552 DOI: 10.1124/jpet.121.000956]

96 **Chang YK**, Choi H, Jeong JY, Na KR, Lee KW, Lim BJ, Choi DE. Dapagliflozin, SGLT2 Inhibitor, Attenuates Renal Ischemia-Reperfusion Injury. *PLoS One* 2016; **11**: e0158810 [PMID: 27391020 DOI: 10.1371/journal.pone.0158810]

97 **Ala M**, Khoshdel MRF, Dehpour AR. Empagliflozin Enhances Autophagy, Mitochondrial Biogenesis, and Antioxidant Defense and Ameliorates Renal Ischemia/Reperfusion in Nondiabetic Rats. *Oxid Med Cell Longev* 2022; **2022**: 1197061 [PMID: 35126806 DOI: 10.1155/2022/1197061]

98 **Tahara A**, Takasu T, Yokono M, Imamura M, Kurosaki E. Characterization and comparison of sodium-glucose cotransporter 2 inhibitors in pharmacokinetics, pharmacodynamics, and pharmacologic effects. *J Pharmacol Sci* 2016; **130**: 159-169 [PMID: 26970780 DOI: 10.1016/j.jphs.2016.02.003]

99 **Abdel-Latif RG**, Rifaai RA, Amin EF. Empagliflozin alleviates neuronal apoptosis induced by cerebral ischemia/reperfusion injury through HIF-1α/VEGF signaling pathway. *Arch Pharm Res* 2020; **43**: 514-525 [PMID: 32436127 DOI: 10.1007/s12272-020-01237-y]

100 **Amin EF**, Rifaai RA, Abdel-Latif RG. Empagliflozin attenuates transient cerebral ischemia/reperfusion injury in hyperglycemic rats *via* repressing oxidative-inflammatory-apoptotic pathway. *Fundam Clin Pharmacol* 2020; **34**: 548-558 [PMID: 32068294 DOI: 10.1111/fcp.12548]

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REVIEW

Evolving spectrum of diabetic wound: Mechanistic insights and therapeutic targets

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Abstract

Diabetes mellitus is a chronic metabolic disorder resulting in an increased blood glucose level and prolonged hyperglycemia, causes long term health consequences. Chronic wound is frequently occurring in diabetes patients due to compromised wound healing capability. Management of wounds in diabetic patients remains a clinical challenge despite many advancements in the field of science and technology. Increasing evidence indicates that alteration of the biochemical milieu resulting from alteration in inflammatory cytokines and matrix metalloproteinase, decrease in fibroblast and keratinocyte functioning, neuropathy, altered leukocyte functioning, infection, *etc.*, plays a significant role in impaired wound healing in diabetic people. Apart from the current pharmacotherapy, different other approaches like the use of conventional drugs, antidiabetic medication, antibiotics, debridement, offloading, platelet-rich plasma, growth factor, oxygen therapy, negative pressure wound therapy, low-level laser, extracorporeal shock wave bioengineered substitute can be considered in the management of diabetic wounds. Drugs/therapeutic strategy that induce angiogenesis and collagen synthesis, inhibition of MMPs, reduction of oxidative stress, controlling hyperglycemia, increase growth factors, regulate inflammatory cytokines, cause NO induction, induce fibroblast and keratinocyte proliferation, control microbial infections are considered important in controlling diabetic wound. Further, medicinal plants and/or phytoconstituents also offer a viable alternative in the treatment of diabetic wound. The focus of the present review is to highlight the molecular and cellular mechanisms, and discuss the drug targets and treatment strategies involved in the diabetic wound.

Key Words: Diabetic Wound; Diabetic Foot Ulcer; Epigenetic mechanisms; Therapeutic agents; Molecular Targets; Phytoconstituents

Core Tip: This paper reviewed molecular pathways and epigenetic mechanisms involved in the pathogenesis of diabetic wounds. The role of microbiota, oxidative stress, inflammatory cytokines, and alteration in the factors involved in normal wound healing process was highlighted. Molecular targets of therapeutic agents, the role of phytochemicals was discussed. The efficacy of several pharmacotherapy, treatment strategies, and recent clinical trials aiming to improve the outcome of diabetic foot ulcers was reviewed.

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INTRODUCTION

The process of wound healing is complex and requires spatial as well as temporal synchronization between different types of cells with specific functions. Hemostasis, inflammatory phase, proliferative phase, re-epithelialization, and remodelling phase are the four major phases of wound healing process, which result in the restoration of functional integrity of tissues[1-3]. Alteration in the microenvironment due to diabetes mellitus (DM) results in a change in the level of oxygen, chemokines, synthesis of growth factors, extracellular matrix, oxidative stress that in turn alter normal cellular recruitment and activation, and induce impaired or delayed wound healing[1,3]. Ageing, genetic disorders, obesity, and metabolic disorders including DM, are responsible for the abnormal wound healing process, that enhances the risk of developing chronic wound[1,3,4]. It was estimated that chronic wounds directly affect the quality of life of about 2.5% population of the United States, and the medicare cost for management of all wounds and the related situation was projected between $28.1 to $96.8 billion[5]. Management of wounds in the diabetic individual is a major clinical and social concern. The hyperglycaemic environment in diabetic people causes impaired and delayed wound healing[4], making the situation more perilous as the number of diabetic people is increasing day by day. It was also found that treatment and management of diabetic ulcers and surgical wounds were the most expensive[5]. The IDF Diabetes Atlas estimated that the prevalence of DM in 2021 was 10.5% (536.6 million people in the 20-79 year age group), which will increase to 12.2% (783.2 million in the 20-79 year age group) by 2045. Global health expenditures related to the management of DM and its complications are expected to reach $966 billion in 2021[6]. It was also predicted that almost half of the adult population (44.7%; 239.7 million of 20-79 years old) were unaware of their diabetic condition. People may develop micro and macrovascular complications during an asymptomatic diabetic state[7]. Impaired or delayed wound healing affects about 25% of diabetic people. A study suggested that 1 in 3 to 1 in 5 diabetic individuals are at risk of chronic non-healing wounds, including diabetic foot (with a very high recurrence rate) in their lifetime[8]. Zhang *et al*[9] estimated that the global prevalence of diabetic foot is 6.3%, which usually affects type 2 diabetic people, older people, and people with a longer duration of DM.

Both the extrinsic and intrinsic factors are responsible for delay in the wound healing process in diabetic patients. Repeated trauma or mechanical stress in the diabetic foot can lead to neuropathy and ischemic situation. Glucose-rich environment results in increased generation of advanced glycation end-products (AGEs) and elevated levels of inflammatory cytokines [*i.e.*, interleukin (IL)-1β and tumor necrosis factor α (TNF-α)] for a persistent period that hinders the normal process of wound healing[10,11]. In turn, hyperglycemia reduces collagen synthesis, growth factor production, macrophage function, angiogenic response, migration and proliferation of fibroblast and keratinocyte, epidermal nerve count, and the balance between extracellular matrix (ECM) component accumulation and matrix metalloproteinase (MMP) induced remodelling [4,12]. The normal wound healing process and effect of the hyperglycemic condition are depicted in Figure 1.

Despite the presence of protocols to standardize care in the diabetic wounds, as well as numerous advancements in scientific research and in clinical fronts, DM remains a problematic situation for wound healing. This paper is an attempt to highlight the mechanistic insights, plausible therapeutic targets, and pharmacotherapeutic approaches, particularly the role of phytochemicals in the management of diabetic wounds, in light of recent shreds of evidence.

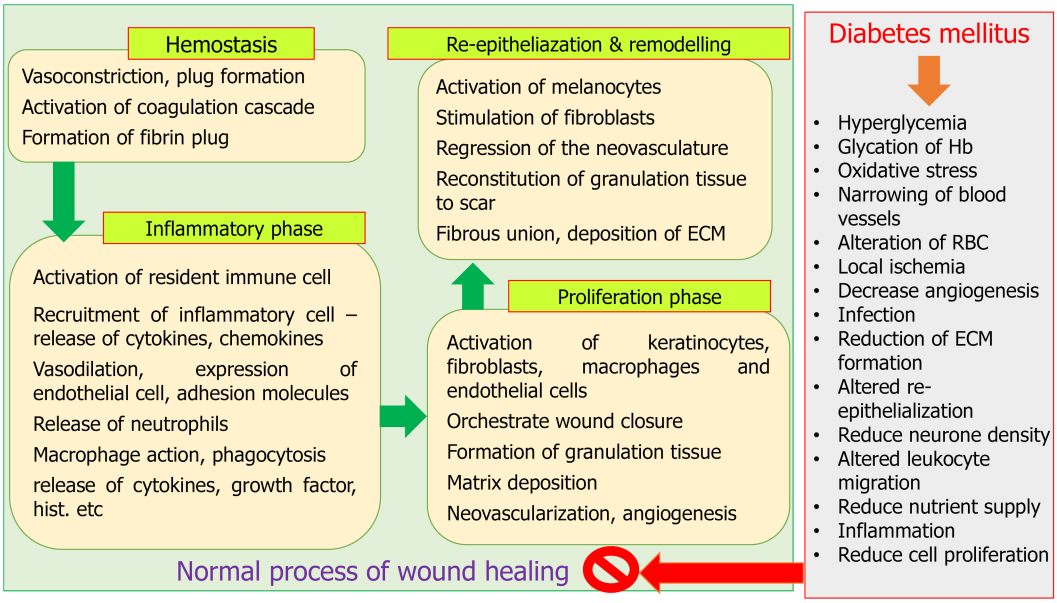

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Figure 1 Normal process of wound healing and effect of diabetes mellitus. ECM: Extracellular matrix.

MECHANISTIC INSIGHTS OF DIABETIC WOUND

Wound healing, being an evolutionary conservation process, restores impaired epithelial barriers through a cascade of events including inflammatory responses, proliferation, cellular migration, angiogenesis, and remodeling of tissues[13]. DM interferes with the normal healing process to provoke non-healing wound, and leads to complications including walking difficulty and infections like septicemia, abscess, cellulitis, osteomyelitis, and gangrene[4]. Acute diabetic wounds with an impaired healing process of unknown aetiology are the first signs of chronic diabetic wounds. Hyperglycemia, hypoxia, chronic inflammation, neuropathy, circulatory dysfunctions, alteration in neuropeptide signalling, and infections impede the diabetic wound healing process. Importantly, due to the heterogeneous nature of the diabetic wound, there exist no clear implications from the pathogenic vantage. The following subsections discuss the potential factors underlying the pathogenesis of diabetic wound.

Molecular implications

Impaired wound healing associated with diabetes remains inconclusive. However, the alterations in cellular factors and biochemical mediators have been believed to be involved in the development and progression of diabetic wound (Figure 2). Factors like hyperglycemia and oxidative stress in diabetic patients result in dysregulated macrophage polarization through modulation of epigenetic codes to delay the process of wound healing[14,15]. Hyperglycemia is implicated in impaired wound closure in diabetic foot ulcers (DFUs), with reduced skin cell function and the formation of atherosclerosis and neuropathy as possible contributors[8]. The development of atherosclerosis leads to alteration in the physiology of endothelial cells along with deprivation of nutrients in the wound site, critically affecting the healing process. Patients with type 1 DM are more prone to macrovascular diseases, especially affecting femoral and metatarsal arteries[16,17]. DM associated early microvascular deficiencies include decreased capillary size, basement membrane thickening, and arteriolar hyalinosis. Thickening of the membrane disrupts the physiological exchanges and causes altered leucocyte migration, and thereby increasing the risk of microbial infections[18,19]. Hyperglycemia disrupts protein translation as well as the migration and proliferation of fibroblasts and keratinocytes involved in the process of re-epithelialization[20-22]. For instance, altered expression of proteins like cytoskeletal keratin proteins (K2/K6/K10) associated with keratinocyte differentiation, and LM-3A32, a laminin-5 α3 chain precursor protein that regulates epithelial cell binding to the basement membrane, was reported in subjects with DFUs[23]. As LM-3A32 is required for the survival and differentiation of keratinocytes, reduction in this protein affects the re-epithelialization process[24]. Interestingly, the expression of mRNA and microRNA was found to be non-significant in diabetic and non-diabetic foot skin fibroblasts[25]. However, fibroblasts from DFUs were reported to exhibit altered morphology, growth factor unresponsiveness, ECM deposition, and reduced proliferation and migration[26-29]. Impaired vasculogenesis and angiogenesis due to deregulation of the growth factors and receptors leads to impaired wound healing. Dysfunctional endothelial progenitor cells (EPC) or reduction in their numbers and transition to proinflammatory EPC phenotypes have been implicated in DM[30,31]. Notably, EPC dysfunction and altered recruitment are contributed by hyperglycemia, chronic inflammation, oxidative

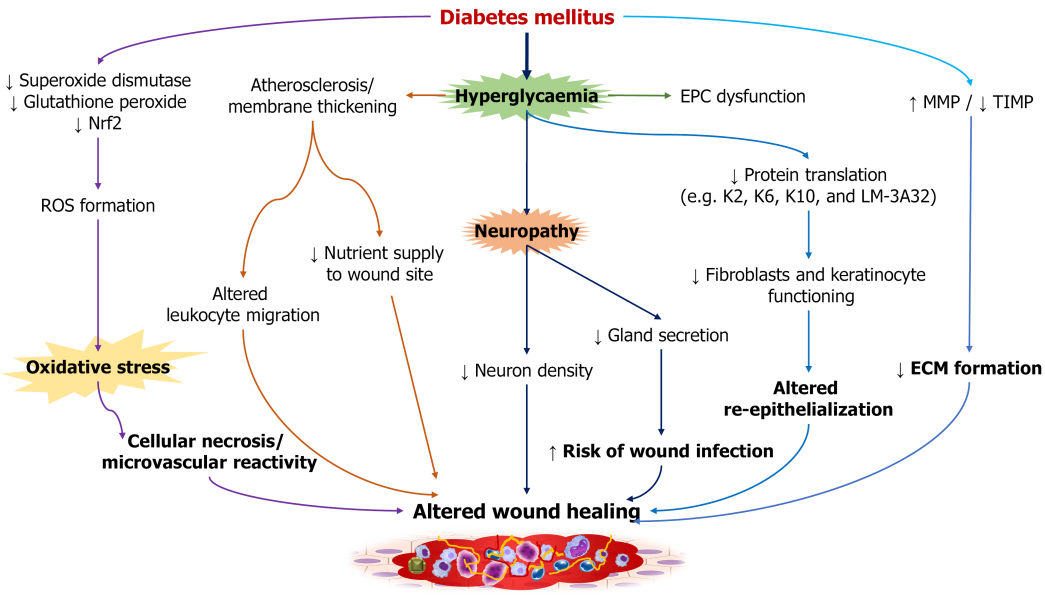

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Figure 2 Altered cellular factors and biochemical mediators involved in the development of diabetic wound. Nrf2: Nuclear factor erythroid factor 2-related factor 2; ROS: Reactive oxygen species; EPC: Endothelial progenitor cell; MMP: Matrix metalloproteinase; TIMP: Tissue inhibitor of metalloproteinase; ECM: Extracellular matrix.

stress, and activation of NADPH oxidase associated with diabetes pathogenesis[32]. Prolonged IL-1β production and reduced expression of peroxisome proliferator-activated receptor-γ are also involved in the impairment of wound healing in DM[33]. Higher expression of MMPs including MMP-1, 2, 8, 9, 14, and 26, and lower expression of their tissue inhibitors were also reported in diabetes[34,35]. In patients with DFUs, it was reported that there is an increase in MMPs and a decrease in tissue inhibitor of metalloproteinase (TIMP)-2, which supports the fact that in a proteolytic environment, diabetic wound fails to heal due to reduction in ECM formation[36]. An increase in MMPs also contributes to matrix degradation, delayed cell migration, and inhibition of collagen deposition[36]. Oxidative stress resulting due to decrease action of enzymes like superoxide dismutase and glutathione peroxidase augment diabetic wound. Overproduction of reactive oxygen species (ROS) from hexosamine, polyol, and AGE pathways affects the later stages of diabetic wound healing, particularly by damaging the peripheral nerves. Consequently, the detrimental effect on the structure, supply, and metabolism of peripheral nerves (neuropathy) increases the risk of DFU development[37]. Hyperglycemia not only contributes to impaired healing but also makes skin prone to injury. Decrease in nuclear factor erythroid factor 2-related factor 2 (Nrf2) in diabetic patients increases the oxidized proteins and ROS generation[38,39]. In Nrf2 knockout mice, delayed wound healing was reported when compared to a $Nrf2^{+/+}$ mice, possibly due to oxidative DNA damage, elevated MMP-9 expression, and lower level of transforming growth factor-beta 1 (TGF-β1) expression[40]. Hyperglycemia also leads to an increase in neutrophil extracellular traps release, which has been implicated in delayed wound healing in both murine and human models[13,41].

The formation of a diabetic wound is also linked to several forms of neuropathy, including sensory, motor, and autonomic. Sensory deficits, for example, cause a loss of protective symptoms, whereas motor neuropathy causes anatomical deformities and ischemic death in the plantar region of the foot [42], and autonomic neuropathy reduces sweat secretion from the glands, making skin dry and increasing the risk of infection and pruritus[43]. Furthermore, neuropathy causes a decrease in neuron density, causing impairment in wound healing. Primarily, diabetic neuropathy occurs in nerves that are dependent on nerve growth factor[8]. Impaired microvascular processes along with autonomic neuropathy and denervation of sympathetic nerves disrupt the blood flow. Cellular necrosis and microvascular reactivity are also triggered by Poly (ADP-ribose) polymerase enzyme *via* oxidative DNA damage[16,44]. In diabetic neuropathy, impairment of C-fibre dependent neurovascular responses leads to abnormality in the release of histamine, substance P, and calcitonin related peptide, thus exhibiting altered vasodilatation in case of stress like pressure or trauma[44]. However, the direct link between neuropathic abnormalities and glucose control is yet to be proven.

Epigenetic mechanisms

Despite the fact that epigenetic pathways have a role in a variety of diabetic complications, evidence of epigenetics in diabetic wound or impaired wound healing is still emerging. The role of microRNA in diabetic wound was first highlighted by a study that revealed up-regulation of miR-503 in plasma

obtained from DFUs and in human umbilical vein endothelial cells (HUVEC). *In vitro* experiment suggested the detrimental effect of forced miR-503 expression on function of HUVEC cells resulting due to impaired migration, proliferation, and formation of blood vessels[45]. Blockage of miR-503 expression showed improvement in angiogenesis in diabetic animals with limb ischemia[45,46]. Another study demonstrated that diabetic mice had a distinct microRNA signature, with differential expression of fourteen microRNAs[47]. Of these, expression of miR-146b was found to be up-regulated by 30 fold. Though miR-21 was up-regulated in diabetic skin, it was reduced in diabetic wound healing. This study suggested the necessity of miR-21 expression for fibroblast migration and miR-21 knock-down results in altered cellular migration. Similarly, another study showed the stabilisation of hypoxia-inducible factor α leads to miR210 expression, which silences the expression of E2F3, an important element of wound healing[46]. This implies the fact that epigenetic changes in miR-210 lead to impaired wound re-epithelialization and reduced proliferation. The involvement of DNA methylation in impaired diabetic wound healing is being studied in many experiments. A study reported inhibition of DNA methyltransferase 1 (DNMT1), an enzyme that transfers a methyl group to the cytosine ring to produce 5-methylcytosine associated with transcriptional repression, suppresses inflammatory signals in bone marrow derived macrophages and also promotes M2-like macrophage formation[48]. This finding was supported by DNMT1 knockdown in db/db mice showing improvement in wound healing[49]. In contrast to that, demethylation of MMP-9 promoter in keratinocytes was reported to be involved in the induction of diabetic wound[50,51]. Apart from the role of DNA methylation in wound healing, it is also associated with metabolic memory, insulin resistance, and other diabetic complications[52,53]. Unlike DNA methylation, histone methylation does not always lead to transcriptional repression. Instead, it silences or promotes transcription based on the target residue and methyl groups. Methylation of histone H3K4 is regulated by SET domain containing protein family, particularly MLL1 that promotes inflammatory gene expression in nuclear factor kappa B-dependent manner[54-56]. A study reported the role of mixed-lineage leukemia-1 (MLL1) is to catalyze H3K4me3 deposition in macrophages during the process of wound healing[57]. Delayed wound healing and reduced pro-inflammatory cytokine generation were reported in a myeloid-specific MLL1 deletion in mice. Monocytes isolated from patients with type-2 DM demonstrated higher MLL1 expression, indicating dynamic regulation of MLL1 expression during diabetic wound healing. In diet-induced obesity model of diabetes, increased expression of histone demethylase (*i.e.*, lysine-specific demethylase 6B or JMJD3) that targets H3K27me3 was seen in wound macrophages[58,59]. Epigenetic regulation of IL-6 expression in neutrophils was believed to be impacted by toll-like receptor (TLR) activation associated with increased H3K27ac, H3K4me3, and acetylated histone H4[60]. Re-epithelialization is also promoted by JMJD3 expression, which induces keratinocyte migration to the wound site[61]. Similar report suggests upregulation of JMJD3 expression on the wound site is necessary for early onset of the wound healing process, which is absent in diabetic wound[62]. However, it will be too oversimplified interpolation as most of these findings are limited to normal wounds; thus, further studies are warranted. Other histone modifications including histone acetylation or deacetylation, histone phosphorylation, histone-arginine demethylation or methylation, and ATP-dependent chromatin remodelling are also being studied for their potential role in diabetic wound healing[62].

Microbiological perspectives

Certainly, pathogenic infections are not directly associated with the pathophysiology of diabetic wounds but are critical from the vantage of impaired wound healing, hospitalization, morbidity, and amputation. However, the role in the initiation of diabetic wound in case of trauma remains unclear. The rapid spreading of infections and high microbial burden exhibit detrimental effect on the wound healing process. Injury to the superficial skin layer allows polymicrobial contamination and colonization, affecting diabetic wound. Particularly, infections like cellulitis, osteomyelitis, and abscesses are of major concern[16]. The advent of high-throughput sequencing technologies like microarray, 16S rRNA sequencing, and whole-genome sequencing have enabled the expansion of diabetic wound microbiome. Diabetic wound has demonstrated higher colonization of *Staphylococcus aureus* and *S. epidermidis*[63]. Another study reported *Staphylococcus*, *Enterobacteriaceae*, and *Pseudomonas* to be the most common colonizers in DFUs[64]. Stratification of DFUs as per infection severity revealed higher bacterial diversity in severely infected DFUs, while *Staphylococcus* and *Streptococcus* were found to be the most abundant in mild-to-moderate DFUs[65]. It was similar to a previous finding that found diverse microbiota (with higher incidence of anaerobic and Proteobacteria infection) in deep chronic ulcers, while *Staphylococcus* was found to be abundant in acute and superficial ulcers[66]. However, contrary to these reports, another study reported *Staphylococcus* spp. to be the primary culture detected in the microbiome in diabetic foot osteomyelitis[67]. A higher prevalence of *S. aureus* colonization in DFUs and intact diabetic skin lead to systemic infection and osteomyelitis[63]. Indeed, in a Nigerian observational multi-center study, it was reported that the presence of osteomyelitis is an important predictor of wound healing in hospitalized patients with DFUs[68]. The expression of proteolytic factors by *Streptococcus* and *Staphylococcus* are believed to be the disruptor of skin barrier. For example, SpeB released by *Streptococcus* leads to cleavage of desmoglein 1 and 3 that causes epidermal barrier damage [69]. The alkaline environment of DFUs contributes to the formation of bacterial biofilm, leading to complex host–microbiome interaction[70]. The formation of bacterial biofilm along with alkaline pH

affects drug action and is responsible for antibiotic resistance[71]. One should appreciate various other factors influencing this intricate microbiome network and its potential correlation with clinical significance. Keeping all this evidence in sight, more longitudinal studies are anticipated for an adequate understanding of the probable relevance of the microbiome to clinical outcomes.

PLAUSIBLE DRUG TARGETS

In recent studies, neuropathy, peripheral vascular disease, and impaired wound healing have all been identified as key contributors to diabetic wounds and have all become critical targets in improving wound healing in diabetic patients. But despite of this well-established knowledge, comparatively fewer treatment options are being implemented in routine practice. The expression of cellular components of participating cells, as well as cytokines, growth factors, and other molecular factors required for coordinating the normal healing process, is impaired in diabetic wounds, and as a result, they are unable to progress in synchrony and are primarily checked in the inflammatory phase. Therapeutic strategy targeting such cellular and molecular pathway could be useful for effective management of diabetic wound.

As a result of elevated blood sugar levels in diabetic conditions, damage to nerve fiber occurs, leading to diabetic neuropathy, which can be sensory, motor or autonomic[72]. Sensory neuropathy can result in one of two outcomes: a painful foot or a foot that is devoid of sensation[12]. Motor neuropathy is accompanied by muscle weakening, atrophy, and paresis. The inability of an intrinsic muscle to keep the foot in its normal state resulting because of weakening of inter-osseous muscles in the foot, which contributes to foot deformity. When the foot deforms, the pressure distribution throughout the foot changes, and aberrant pressure develops at various locations on the foot[73]. Keratosis and callus development occur as a result of repeated pressure, which leads to damage to callused areas and induces ulcer formation beneath the callus that further causes cracks on the foot[74-77]. Malfunctioning of the sympathetic nerves supplying the sweat glands in the foot reduces the sweat and moisture in the feet, which leads to the development of cracks[78]. Inflammation, necrosis, and ulceration result when an unnoticed injury is combined[16,79]. Currently, duloxetine, anticonvulsant pregabalin and opioid tapentadol are being prescribed for diabetic peripheral neuropathy. Besides these, a substance like α-lipoic acid has shown effectiveness in delaying or reversing peripheral diabetic neuropathy through its multiple antioxidant properties[80]. Neuropeptides such as substance-P and neuropeptide-Y are also been found to be effective in diabetic neuropathy and associated wound[81,82].

Peripheral vascular dysfunction is another major cause of diabetic wound in a majority of diabetic patients. The wound healing process in the diabetic condition is hampered by the altered physiological response due to glycation of hemoglobin, alteration of the red blood cell membrane and narrowing of blood vessels which cause the decreased supply of nutrients and oxygen to tissues[12]. The development of atherosclerotic plaque in diabetic patients also leads to the development of non-healing wound. Therefore, pharmacotherapeutic agents like antioxidant phytochemicals that can avert oxidative stress and formation of AGEs could be useful in treating diabetic wound.

Bacterial infection is one of the most common causes of wounds, and diabetic individuals are more vulnerable to it because of delayed wound healing and immunosuppression[83,84]. The biofilms created by microorganisms protect them from antimicrobial agents and the immune system while also interfering with the healing process, which is one of the most prevalent reasons for amputation of lower limbs in diabetic wounds[14]. Therefore, empiric therapy should include broad-spectrum antibiotics. In recent times, drug resistance is a bigger problem and several drugs are in use in the treatment of DFU.

Neutrophils, monocytes, macrophages, keratinocytes, fibroblasts, T cells, B cells, mast cells, and endothelial cells are all involved in wound healing and are responsible for the formation and modulation of pro-inflammatory cytokines and growth factors such IL-1, TNF-α, IL-6, vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and TGF-β. Hyperglycemia and oxidative stress lead to dis-regulation of these cells, resulting in delayed wound healing[85-87]. Increased amounts of pro-inflammatory cytokines cause an inflammatory cascade to be disrupted, resulting in hyper-inflammation and insulin resistance. These also lead to reduced angiogenesis and microvascular issues, impaired macrophage and neutrophil function, impaired keratinocyte and fibroblast migration and proliferation, and impaired growth factor generation[15,88-90]. Many of these cells play a vital role in the immune response, which is also important for wound healing. Various chemokines whose expression can regulate the function of immune cells have the potential to enhance wound healing. Mast cells with close coordination with macrophages, endothelial cells, and fibroblasts, play a key role in matrix remodeling and disrupt the balance of pro- and anti-angiogenic molecules in wound tissues, affecting angiogenesis and vascular regression in the proliferative and remodeling phases, respectively[91-93]. As a result, mast cell degranulation inhibitors such as disodium cromoglycate, quercetin, and luteolin may be promising options for improving diabetic wound healing [92]. Heat shock proteins (HSPs) aid wound healing by attracting dermal fibroblasts, stimulating cell proliferation and keratinocyte differentiation, reducing oxidative stress, ameliorating actin microfilaments, aiding endothelial cell migration, and enhancing pro-collagen synthesis and protein

homeostasis[94,95]. Reduced levels of HSPs and their downstream components TLR4 and p38-MAPK (mitogen-activated protein kinases) in diabetic patients are responsible for the slowed healing process [96]. Therefore, targeting this as a therapeutic target could be useful in diabetic wound conditions.

Growth factors are biologically active polypeptides that play an important role in the onset and maintenance of wound healing[97]. In diabetics, any change, *i.e.*, down-regulation of growth factor receptors and rapid degradation of growth factors, causes wound healing to be delayed. Factors such as VEGF, IGF, TGF-β, KGF24, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), TNF-α, and IL-6 are significantly reduced in diabetes patients, and several of them have been demonstrated to significantly enhance wound healing in many studies. Growth factors that cause a molecular alteration in the wound micro-environment may help patients with non-responsive wounds. PDGF is a major serum mitogen that promotes fibroblast proliferation, matrix formation, and connective tissue maturation[98]. They attract fibroblasts and inflammatory cells and aid in the production of glycosaminoglycans, proteoglycans, and collagen. During the healing process, it is a critical mediator in fibroblast migration and proliferation, the formation of granulation tissue proteins and provisional extracellular matrix, and angiogenesis[99]. The expression of PDGF and its receptors is reduced in diabetic wounds, and many clinical studies employing PDGF have shown improved healing time[100,101]. It is indicated for the treatment of infections that have spread to deeper subcutaneous tissues or beyond areas with a sufficient blood supply[102]. Another growth factor, bFGF, has a stimulatory effect on fibroblast growth and differentiation, as well as the proliferation of vascular smooth muscle cells, endothelial cells, ECM metabolism, growth, and movement of mesodermally derived cells, all of which speed up the formation of granulation tissue and promote wound healing [103]. Angiogenesis, cell proliferation, migration, differentiation, neo-vascularization, re-epithelialization, and collagen disposition were all stimulated by the clinical application of bFGF, all of which contribute to wound healing[104]. It promotes mesodermal cell chemotaxis and extracellular matrix growth and expedites both acute and chronic wound healing, which gives a scar-free cure[105]. VEGF is a potent angiogenic cytokine that has a substantial impact on healing and promotes rate-limiting processes in vasculogenesis and angiogenesis[106]. Low VEGF levels cause impaired wound healing and aberrant VEGF receptor patterns in diabetics. Decreased VEGF mRNA levels, increased VEGF receptor (VEGFR)-1 Levels, and decreased VEGFR-2 Level are some of the key causes of wound non-healing[107]. In diabetic wounds, VEGF leads to an increase in capillary density, which enhances blood perfusion and metabolism in the wounded tissue[108]. VEGF causes an increase in capillary density, which improves blood perfusion and metabolism in the wounded tissue. This leads to the facilitation of the supply of oxygen and nutrients to assist the growth and function of reparative cells. It is the primary regulator of wound revascularization and permeability and participates in the formation of granulation tissue. On binding with the EGF receptors, EGF causes an increase in epidermal cell, cell motility, cellular migration, mesenchymal regeneration, angiogenesis and cell proliferation[109]. Application of EGF into the wound site results in a greater pharmacodynamic response in terms of granulation tissue growth and wound closure. IGF-1 promotes wound healing by assisting in cell granulation and re-epithelisation, promoting endothelial cell chemotaxis and keratinocyte and fibroblast proliferation, while lower levels of both IGF-1 and TGF-β in wound tissue cause wound healing to be delayed[110, 111]. TGF-β attracts and stimulates inflammatory cells such as neutrophils, macrophages, lymphocytes, keratinocytes, and fibroblasts, as well as the synthesis of growth factors, which speed up vascularisation, angiogenesis, and ECM synthesis while slowing down ECM degradation[112]. Therapeutic agents or strategy, which can ameliorate this positively, are useful in diabetic wound condition. Several drugs and phytoconstituents have shown their positive effect in diabetic wounds targeting these biomolecules. The use of platelet-rich plasma (PRP), EGF, PDGF, and FGF has shown promising effects in the treatment of diabetic wound in a better way.

MMPs are a class of endopeptidase that play a key part in wound debridement, as well as angiogenesis, epithelialization, and extracellular matrix remodeling[113]. Matrix proteins such as collagens, basement membrane collagens, proteoglycans, elastin, and fibronectin are digested by the MMPs. TIMPs form a complex with MMPs, limiting interaction with the active site. A balance between MMPs and TIMPs is required for wound healing, which is impaired in diabetes patients[114]. Increased protease activity caused by high MMP levels in diabetic wounds causes tissue damage and slows down normal repair processes[115]. This is due to altered MMP expression and decreased TIMP expression in diabetic conditions, which results in high levels of pro-inflammatory and pro-fibrotic cytokines due to increased inflammatory cell activation and invasion, and indirectly affects MMPs through the formation of advanced glycation products which leads to the loss of growth factors, receptors, and matrix proteins essential for wound healing[111,114]. Drugs, therapy that is useful in diabetic wounds are found to act by enhancing collagen synthesis, decreasing inflammatory cytokines, AGEs *etc*. Further, identifying the therapeutic agents that can inhibit MMPs, *i.e.*, MMP-1, MMP-8, and MMP-9 could be important in the management of diabetic wounds.

Autologous stem cells capable of self-renewal and multi-lineage differentiation have been used in diabetic wound. Clinically, bone marrow-derived mononuclear cells and mesenchymal stem cells are the most successful stem cell therapies[116,117]. Besides these, several other targets to promote diabetic wound healing include stimulation of nitric oxide production and up-regulation of endothelial NO synthase and nitric oxide (NO) expression[118-120], a decrease of AGE receptors[121], collagen

generation and epitheliazation *etc*[122-124]. Figure 3 represents plausible drug targets for diabetic wound.

MANAGING DFU: PHARMACOTHERAPY

Hyperglycemic environments worsen the wound situation and delay the wound healing process, thus controlling blood sugar levels is important for the wound healing process. Several antidiabetic medications [*i.e.*, insulin, metformin, sulfonylureas, dipeptidyl peptidase-4 (DPP-4) inhibitors, thiazolidinediones] found effective in controlling not only blood sugar levels but also promoting the healing of a wound. Metformin was found to be effective in wound healing, which may act by improving epidermis and deposition of collagen, and increasing TGF-β production that may link with angiogenesis process, inflammatory reaction, re-epithelization, and remodeling process. DPP-4 inhibitors may act by increasing the generation of SDF1α, which is crucial for wound repair process[11, 125]. Drugs like simvastatin (enhance VEGF and NO content at wound site) and phenytoin (enhance VEGF and FGF in wound area) have also shown some positive effects in wound healing[11,125].

Infection caused by microbes is a major problem in DFU. Antimicrobial therapy targeting Gram -ve, Gram +ve, anaerobic bacteria, and certain fungi, contributing to the pathophysiology of DFU, is also a key approach. Povidone iodine solution (10%), chlorhexidine, acetic acid (5%), and silver compounds can be used to treat the mild wound. Antibiotics like amoxicillin + clavulanate, ampicillin, dicloxacilline, cephalosporin (*i.e.*, cephalexin, cefoxitin, ceftriaxone, and ceftazidime), quinolones (*i.e.*, ciprofloxacin, levofloxacin), metronidazole, and clindamycin used to cure moderate DFU[126]. Drugs like piperacillin/tazobactam + clindamycin, meropenem, imipenem, ertapenem, ampicillin + sulbactam are used to cure severe infections caused by different Gram +ve and Gram -ve bacteria, anaerobes, MRSA, *P. aeruginosa*, while vancomycin, linezolid, and daptomycin can cure MRSA[11]. Cefepime, ceftazidime, meropenem, and piperacillin/tazobactam are recommended to use against *P. aeruginosa;* Metronidazole and clindamycin are used to manage infection caused by anaerobes, while osteomyelitis can be managed with quinolones and linezolid. Further, the effectiveness of different drugs like penicillin, cephalosporin (ceftazidime, ceftriaxone, and cefuroxime), dicloxacillin, vancomycin aztreonam, cefalotin, clindamycin, cefoxitin, gentamicin, imipenem, piperacillin/tazobactam, imipenem, metronidazole, amikacin, levofloxacin, and cefalotin against microbes contributing in DFU infection also established[126]. Dressings with hydrogels, acrylics, hydrofibers, films, hydrocolloids, calcium alginates, polyurethane foam and ciprofloxacin-loaded calcium alginate wafer are also used to control the infection[126,127].

Debridement (elimination of bacterial biofilm and necrotic tissue from wound site), offloading (complete/partial removal of pressure), PRP, EGF, PDGF, FGF, oxygen therapy, negative pressure wound therapy (NPWT), low-level laser therapy (LLLT), extracorporeal shock wave therapy (ESWT), bio-engineered skin substitutes/ soft tissue substitutes (amniotic membrane, autologous stem cell therapy, bi-layered bio-engineered skin substitute, human fibroblast-derived dermis, porcine small intestine submucosa), and maggot therapy are also used successfully to cure DFU[127-129].

In clinical practice, control of blood glucose level, periodical foot screening, patient education, use of therapeutic footware by susceptible people, prophylactic arterial revascularization are important in prevention of DFU[130]. Off-loading in different grades of DFUs (grade 1B, 1C), and diagnosis of diabetic foot osteomyelitis are recommended in grade 1B, 1C, 2C DFU using different screening methods by Society of Vascular Surgery[130]. Surgical debridement to remove necrotic and devitalized tissue, use of proper dressing to create and maintain moist environment, reducing plantar pressure and shear stress (off-loading), vascular assessment in patient with peripheral arterial disease are the vital part of wound care in diabetic people[130,131]. Microbial infection is a major concern in DFU, and in such cases, use of antimicrobial agents is advised conserving extent of infection[131]. A number of adjuvant therapy as discussed in previous section are also used for effective wound healing. Further, adequate glycemic control is important also in acceleratd healing of DFU's[130,131].

RECENT APPROACHES IN CLINICAL TRIALS

Several treatments /medicines have been successfully investigated clinically for their beneficial effects on the diabetic wound, especially in DFUs which was tabulated in Table 1[132-159].

PRP is an important treatment approach investigated in DFU. A systematic review with meta-analyses of 10 studies reported that PRP promotes chronic diabetic wound healing (RR = 1.32; 95%CI: 1.11-1.57, I^2 = 15%) by reducing the volume and time of wound healing[160]. PRP may act *via* promoting the proliferation of wound cells, upregulation of cyclin A and cyclin-dependent kinase 4 proteins, modification of macrophage phenotype, reduction of TNF-α, enhancement of TGF-β and VEGF, increased secretion of fibroblast of collagen type I and III[161].

Table 1 Clinical trial of few therapeutic agents/approaches in the management of diabetic wound

Ref.	Type	Drug/ product /approach investigated	Type and number of participants	Type of wound	Observation
[132]	Homeopathic medicine	Silicea, Sulphur, Lycopodium, Arsenic album, Phosphorus	Observational study, 156 patients	DFU	Ulcer assessment score reduced significantly ($P < 0.05$) after treatment. Silicea, sulphur ($n = 11$), lycopodium, arsenic album, phosphorus were found more effective. Although, the effect of homeopathic therapy alone is difficult to establish
[133]		Silicea	Observational study, 22 patients	DFU	Positive and encouraging result of silicea in ulcer healing was reported. DFU assessment score was measured, and mean symptom scores at the end of the treatment were found to reduce significantly ($P < 0.05$)
[134]	Herbal Products	ON101 cream (contain extract of *Plectranthus amboinicus* and *Centella asiatica*)	Phase 3 RCT, 236 participants	DFU	Incidence of complete healing in ON101 and comparator group was 60.7% and 35.1% respectively. Although, the number of adverse events in the ON101 group was 7 *vs* 5 in the comparator group. ON101 produced a better healing effect compared to absorbent dressing alone
[135]		Intravenous Semelil (a naive herbal extract)	RCT, 25 participants	DFU	Mean foot ulcer surface area reduced significantly in semelil (i.v. route) group and the average wound closure in semelil group was significantly more than control group (64% *vs* 25%, $P = 0.015$). Semelil in combination with conventional therapy showed better effect than conventional therapy
[136]		Olive oil	Double blind RCT, 34 participants	DFU	Degree of ulcer, color, surrounding tissues, the status of ulcer and ulcer drainages were evaluated after topical application of olive oil. Complete ulcer healing in the treatment group was significantly better than the control group (73.3% *vs* 13.3%, $P = 0.003$). Olive oil treatment significantly reduced ulcer area and ulcer depth. Olive oil in combination with routine care was better than routine care alone
[137]		Polyherbal formulation (contain *G. glabra*, *M. paradisiaca*, *C. longa*, *P. odaratissimus*, *A.e vera*, *C. nucifera oil*)	Open label, phase III, comparative study, 40 participants	DFU	Polyherbal formulation was found effective similar to that of standard silver sulphadiazine cream
[138]		Semelil (ANGIPARS™, contain *Melilotus officinalis*)	Clinical study, 10 participants	DFU	ANGIPARS™ was found effective in reduction of wound size by at least 50% during 8 wk period
[139]	PRP	PRP gel	Single-arm clinical trial, 100 participants	DFU	PRP therapy (2 mL/cm^2 of ulcers) was found highly effective in the treatment and healing of non-healing chronic DFUs
[140]		PRP	Prospective RCT, 20 participants	DFU	Wound healing time was estimated as 8 wk which is superior to the control group. People treated with PRP it found more effective in wound healing
[141]	Human EGF (hEGF)	Recombinant hEGF	Prospective, open-label trial, crossover study, 89 participants	DFU	Wound healing was noted within an average of 46 d in patients who were treated with 0.005% EGF twice a day. Topical application of hEGF combined with hydrocolloid dressing showed promoting healing effect in chronic DFU
[142]		Regen-D 150 (hEGF gel-based product)	RCT, 50 participants	DFU	Complete ulcer healing was detected in 78% population against 52% population in the placebo group. Collagen and fibroblasts were significantly developed in the treated group. The application of hEGF can be helpful to promote wound healing and in preventing leg amputations
[143]	PDGF	rhPDGF	RCT, 50 participants	DFU	Wounds contracted more in rhPDGF-treated group compared to the control group (38.55% *vs* 12.79%; $P \leq 0.001$). Dressing with rhPDGF was found more effective and promoted safe wound healing
[144]		PDGF gel	RCT, 29 participants	Diabetic lower extremity ulcer	100% of ulcers were healed in subjects who received PDGF compared to 76.4% of wound healing in placebo group. The study confirms the effectiveness of PDGF gel
[145]	FGF	bFGF	Double-blind RCT, 150 participants	Non-ischaemic diabetic ulcer	Wound cure rate in 0.001% bFGF, 0.01% bFGF and control group was 57.4%, 66.7% and 46.8%. The area of the ulcer was also significantly decreased in bFGF treated groups. bFGF accelerates wound healing in diabetic people
[146]	Oxygen therapy	Topical oxygen therapy	RCT, 145 participants	DFU	A significant decrease in wound area was reported in the topical oxygen therapy + standard care group (70%) compare

					to the standard care group (40%) Addition of topical oxygen therapy with standard care facilitates wound closure in a better way
[147]		Hyperbaric oxygen therapy	RCT, 75 participants	Chronic DFU	Complete healing of ulcer index was reported in 52% of participants who received hyperbaric oxygen therapy after 1 year, which was 29% in the placebo. Adjunctive treatment with hyperbaric oxygen therapy may facilitate the healing of foot ulcers
[148]	NPWT	NPWT	Prospective randomized study, 55 participants	DFU	Granulation tissue formation (91.14% *vs* 52.61%, $P < 0.001$) and a decrease in the size of ulcer size (40.78% *vs* 21.18%, $P = 0.008$) were reported in the NPWT group after 14 d. Duration of hospital and time for complete coverage of the wound with granulation tissue was significantly less in the NPWT group. NPWT led to an early decrease in the size of the ulcer, formation of more granulation tissue, and wound healing
[149]		NPWT	RCT, 55 participants	DFU	The rate of ulcer healing was found higher in the NPWT group (*P*-value 0.01). NPWT leads to a higher rate of healing, and causes a significant decrease in ulcer surface area, size, and depth of the wound, reducing the risk of amputations
[150]	Phototherapy	LLLT	RCT, 23 participants	DFU	Ulcers size reduced significantly in 4th week in LLLT group ($P = 0.04$). More patients healed completely in LLLT group compared to the placebo group. Meantime of complete healing in patients treated with LLLT was 11 wk *vs* 14 wk in placebo patients. LLLT promotes the healing process of chronic DFU, and reduces the time required for wound healing
[151]		LLLT	RCT, 56 participants	DFU	Increment in total hemoglobin was more using the highest intensity configuration compare to the lower intensity setup in patients with DFU. A decrease in the very-low frequency/low frequency ratio, slightly more than the highest intensity in DFU people was observed. LLLT was found to increase blood flow and regulation of the autonomic nervous system in patients with DFU
[152]	ESWT	ESWT	Single-blinded RCT, 38 participants	DFU	Patients received shock wave therapy 2/week for a total of 8 treatments. Average healing time was lower in ESWT-group when compared with the control group (64.5 *vs* 81.17 d, $P < 0.05$). AT 20 wk, 54% of ulcer healed completely in ESWT-groups compared to 28.5% in the control group
[153]		ESWT	Prospective RCT, 23 participants	DFU	At 7 wk, the mean reduction in ulcer area was 34.5% (CI, 0.7-68.3) in the ESWT group and 5.6% (CI, -42.1-53.3) in the control group. ESWT also enhances tissue oxygenation
[154]	Stem cell therapy	Topical application of MSC	Clinical case study of three patients	Neuropathic DFU	MSCs at low doses enhance the re-epithelialization of DFU. MSCs may start early to reduce overall wound closure time
[155]		HUCMSCs	RCT, 56 participants	DFU	Patients in HUCMSCs (endovascular infusion and injection around the foot ulcer) experienced greater and betterment in skin temperature, transcutaneous oxygen tension, ankle-brachial pressure index, and claudication distance. Three months after treatment significant enhancement in neovessels, and complete or gradual ulcer healing was observed in the experimental group
[156]	NO generating approach	EDX110 (nitric oxide generating medical device)	RCT, 135 participants	DFU	At 12 wk, EDX110 use resulted in 88.6% reduction in median wound area compared to 46.9% for the control group ($P = 0.016$). EDX110 was found to improve foot wound healing in diabetic people significantly by reducing the ulcer area
[157]	Other Approaches	Bemiparin (low MW heparin)	RCT, 70 participants	DFU	In bemiparin group, the ulcer improvement rate was 70.3% compared to 45.5% in the placebo group. Though, complete healing rates found similar in both groups at 3 mo were, as were the number of adverse events. Bemiparin is better than a placebo in the management of DFU and has few side effects
[158]		Honey dressing	RCT, 348 participants	DFU	In 75.97% of cases wound healed completely after honey dressing in comparision to 57.39% of case in the saline dressing group. The homey dressing also reduced the median wound healing time (18.00 d) compare to the control group (29.00 d). Honey is an effective dressing substance compared to conventional dressings
[159]		Omega-3–rich fish skin grafts	RCT, 49 participants	DFU	At 12 wk, 67% of foot wounds were completely closed compared with 32% in the standard care group. Study findings indicated that fish skin graft is useful in the treatment of chronic DFUs that do not heal with standard treatment

DFU: diabetic foot ulcers; RCT: randomized controlled trials; PRP: platelet-rich plasma; hEGF: Human endothelial growth factor; rhPDGF: Recombinant human platelet-derived growth factor; PDGF: platelet-derived growth factor; bFGF: Basic fibroblast growth factor; NPWT: Negative pressure wound therapy; LLLT: Low-level laser therapy; ESWT: Extracorporeal shock wave therapy; MSC: Mesenchymal stromal cell; HUCMSCs: Human umbilical cord mesenchymal stem cells; NO: nitric oxide.

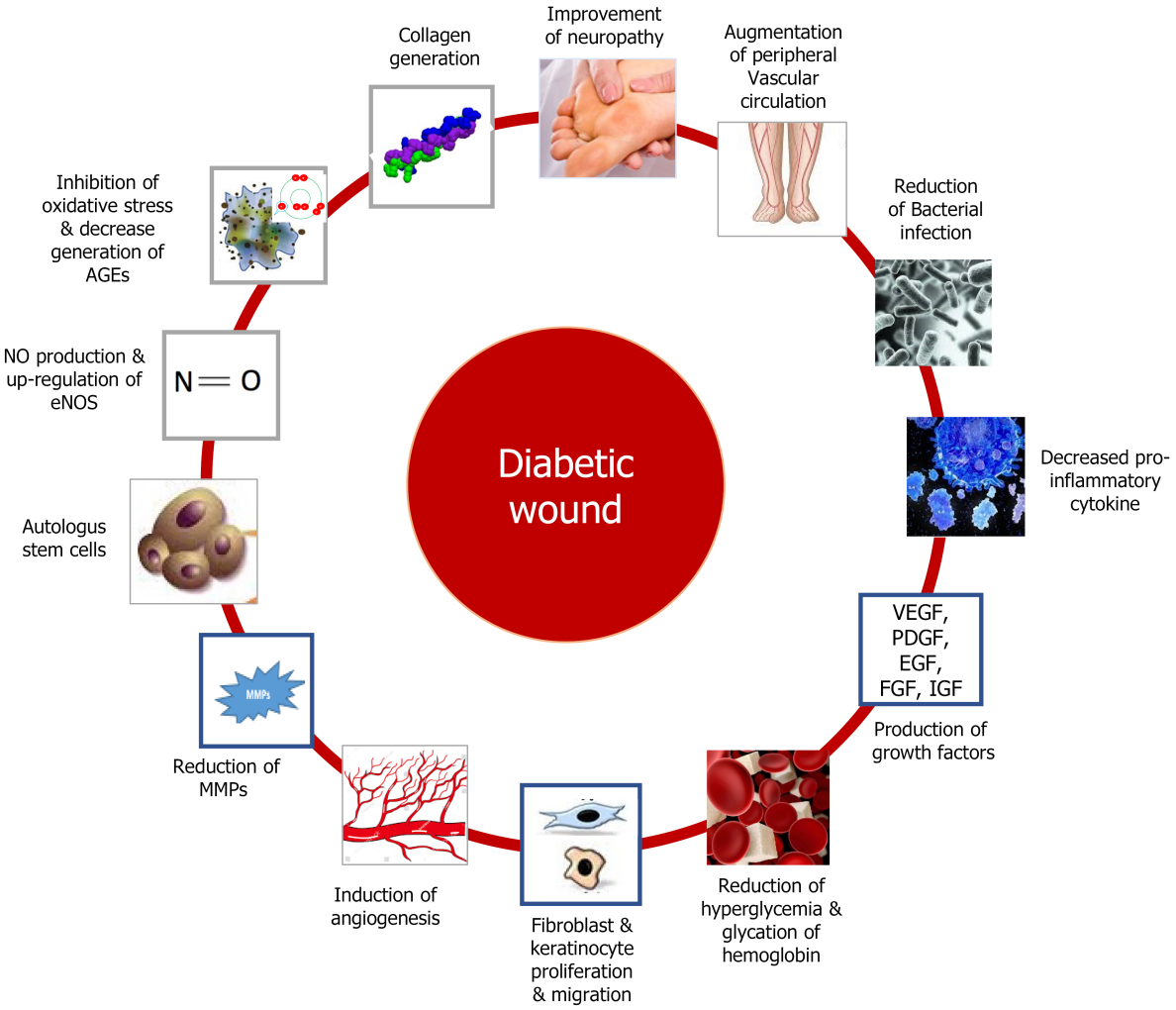

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Figure 3 Plausible drug targets for diabetic wound. AGEs: Advanced glycation end-products; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; IGF: Insulin-like growth factor.

A systematic review of 26 randomized controlled trials (RCTs) examined the usefulness of different recombinant proteins and growth factors in the treatment of DFU. EGF can be used as intralesional injection, topical cream, or gel. This meta-analysis reported that EGF significantly improves wound healing and the study failed to find a significant effect of EGF in reducing the risk of amputation[162]. Decreasing the level of circulating C-reactive protein, decreasing NFκB1, TNF-α, and IL-1a expression, and increasing the wound expression of platelet-derived growth factors (PDGF)-B, cyclin-dependent kinase 4, P21, TP53, angiopoietin 1, collagen 1A1, MMP-2, and TIMP-2 after the treatment with hEGF may be linked to its beneficial effect[161]. Recombinant human PDGF (rhPDGF) also exhibits their beneficial effect in several clinical studies[143,144], but few clinical studies also failed to find statistically significant effects of rhPDGF compared to the control group[163,164] when used to cure DFU. The effectiveness of PDGF may be related to the promotion of fibroblast and leukocyte migration, and synthesis of extracellular matrix[161]. FGF, a family of cell signaling proteins, is found to stimulate angiogenesis, induce fibroblasts proliferation, and promote wound healing. About 23 subtypes of FGF from 7 subfamilies were identified[165]. Some clinical studies have reported the beneficial effect of FGF in diabetic wound[145], some studies failed to report the advantageous effect of FGF over the placebo group[166], while clinical studies reported the beneficial effect of combined use of human EGF and acidic FGF on wound healing in the later stage[167]. Very few clinical studies evaluated the effectiveness of granulocyte-colony-stimulating factor, topical telbermin, epoetin-β, talactoferrin, TGF-β2 in

the diabetic wound. But the results are inconsistent[162].

Oxygen therapy is found to improve cell metabolism and energy, decrease proinflammatory cytokines and ROS, and promote the synthesis of matrix and wound repair[161]. Few clinical studies confirmed the beneficial effect of hyperbaric as well as topic oxygen therapy in promoting diabetic wound[146,147]. A systematic review and meta-analysis of 20 RCTs and 1263 trials analysed that hyperbaric oxygen therapy confer benefits in DFU treatment by increasing the healing of ulcers (relative risk, 1.901; 95%CI: 1.484-2.435, $P < 0.0001$), reducing healing time, and also decrease the risk of major amputation[148].

It was evaluated that NPWT results in macro- and micro-deformation that stimulate different wound healing cascade like promotion of tissue granulation, epithelialization, proliferation of vessel, neo-angiogenesis, pro-angiogenic condition, removal of surplus extracellular fluid, anti-inflammatory effect, increase expression of VEGF, FGF2, modification of circulating micro-RNAs, alteration of DNA methylation of genes linked with wound repair[161,168]. Liu *et al*[169] in their study (meta-analysis of 11 RCTs) concluded that NPWT is a safe, cost-effective and effective strategy in the treatment of DFU, while Liu also evaluated 11 RCTs and found that compared with wound dressings NPWT may enhance the proportion of wounds healed, decrease the time of postoperative foot wound healing[170].

LLLT was found to increase blood flow and regulation of the autonomic nervous system in patients suffering from DFU[151]. Reduce wound inflammation, enhance fibroblasts and angiogenesis[161], which may play an important role in diabetic wound healing. A metanalysis of 13 RCTs that included 413 patients concluded that LLLT significantly enhanced complete healing rate (RR = 2.10, 95%CI: 1.56-2.83, $P < 0.00001$), decreased wound ulcer area, and reduce mean healing time of wound healing in patients suffering from DFU[171].

Huang *et al*[172] performed a meta-analysis of 8 RCTs and concluded that ESWT treatment reduced wound surface area in greater proportion, enhances re-epithelialization and can reduce treatment inefficiency. ESWT is useful as an adjuvant strategy in the management of DFUs, which can improve the complete wound cure rate and reduce the healing period of DFUs. ESWT may enhance the angiogenesis process, decrease macrophage number, and enhance the production of macrophage of growth factors from macrophages that help in the healing of wound[161].

In light of current evidence, it can be suggested that stem cell-based therapy (delivery through both local and systemic route is effective to heal DFU and considered a promising regenerative medicine, and mechanisms of stem cell therapy include improved angiogenesis, decrease inflammation, ameliorating neuroischemia, improved collagen deposition, *etc.*[173].

Wound healing in diabetic people can be promoted by providing endogenous or exogenous NO. Products (*i.e.*, patches/ matrices) that release NO are used to treat diabetic wounds by different mechanisms like enhancement of angiogenic activity, endothelial cell proliferation, conferring antimicrobial substances, and promoting cell migration to the injured site[174]. Only a few clinical studies have reported the beneficial effects of NO-releasing devices, and several products are in the clinical trial.

Homeopathic medicines (like silicea, sulphur, lycopodium, and arsenic) were also investigated in the clinical trial and concluded that homeopathic medicine may be useful in the management of diabetic wounds[132,133]. Several other products, like honey, fish skin grafts, *etc.*, were also clinically investigated for their beneficial effects against DFU[158,159]. Further, several herbal products were also successfully investigated in the treatment of diabetic wound[134,135,136-138].

MEDICINAL PLANTS AND PHYTOCONSTITUENTS IN DIABETIC WOUND

Medicinal plants and phytoconstituents always represent an important, effective and alternative treatment strategy to cure diseases. Phytochemicals have showed their effectiveness in different diabetes complications. Anti-inflammatory mechanism of phytochemicals is considered important in the management of diabetes wound. Epigallocatechin gallate in pre-clinical investigation was found to reduce reduced levels of IL-1β, TNF-α and IL-6, producing inhibition of Notch signaling and accumulation of macrophage at a wound site. Kaempferol, is an important dietary flavonoid found to exert different pharmacological activities, including antioxidant, anti-inflammatory and cardioprotective activity. An ointment containing Kaempferol was found effective in diabetic excisional and non-diabetic incisional wounds in experimental animals[175]. Flavonoids an important class of phytoconstituents, exerted anti-inflammatory and antioxidant effect, and also enhances angiogenesis and re-epithelialization. Preclinical trials found the effectiveness of isoliquiritin, isoflavonoid, naringenin, dihydromyricetin, dihydroquercetin, quercetin, hesperidin, kaempferol, proanthocyanidins, icariin, puerarin, rutin, genistein, luteolin, rutoside, silymarin, daidzein, genistein, and epigallocatechin gallate to cure wound[174]. Flavonoids positively regulate MMP-2, MMP-8, MMP-9, MMP-13, Ras/Raf/MEK/ERK, PI3K/Akt, and NO pathways. Phytochemicals are found to reduce oxidative stress, expression/release of proinflammatory/inflammatory cytokines, *i.e.*, TNF-α, IL-1β, IL-6, NF-κB and upregulate IL-10 and antioxidant enzymes. Flavonoids also act on macrophages, fibroblasts and endothelial cells by facilitating expression/release of TGF-β1, VEGF, angiopoietin, tyrosine kinase with immunoglobulin and epidermal growth factor homology domains, and small mothers against

decapentaplegic 2 and 3[176]. Oguntibeju[177] in his paper highlighted different medicinal plants like *Rosmarinus officinalis*, *Carica papaya*, *Radix rehmanniae*, *Annona squamosa*, *Catharanthus roseus*, *Centella asiatica*, *Acalypha langiana*, *Hylocereus undatus*, *Punica granatum*, *Aloe vera*, and *Martynia annua* that has been investigated in the treatment of diabetic wound. Benefits of the plants may link to different mechanisms like an increase in fibroblast cell, fibroplasia, increase in collagen formation, enhancement of tissue regeneration, angiogenesis, antimicrobial, anti-inflammatory and antioxidant effect. A recent clinical study established the effectiveness and safety of nano-hydrogel embedded with quercetin and oleic acid when used in the management of lower limb skin wound in diabetic patients. The formulation effectively treated the wound and reduce the wound healing time compared to the control group[178]. Infections caused by different microbes like *Staphylococcus aureus*, *Streptococcus* β-hemolytic, *Pseudomonas aeruginosa*, *Peptostreptococcus spp.*, *Proteus spp.*, *Prevotella spp.*, *Bacteroides spp.*, *Clostridium spp.* and anaerobes are posing a serious situation in diabetic people[126]. Medicinal plants and phytochemicals with antimicrobial activity may also play an important role in the management of diabetic wound. Several plants have shown their potential against the microbial strain responsible for infection in the diabetic wound[126]. Formulation designed with *Momordica charantia*, *Actinidia deliciosa*, *Aloe vera*, *citrus fruits*, *Sida cordifolia*, *Nigella sativa*, *Curcuma longa*, and *Azadirachta indica* has shown their potential in the treatment of diabetic wound[126]. Isoflavones isolated from plant sources were also found to be effective against DFU bacteria[179] Phytofabricated silver nanoparticles (*Aerva lanata* reduced silver nanoparticles) at 20 μg/mL were found highly effective against multi antibiotic-resistant DFU isolates like *E. coli*, *P. aeruginosa*, *S. aureus, S. subtilis*. Identified phytochemicals of *A. lanata* include rutin, quercetin, kaempferol, gallic acid and ellagic acid[180]. The use of phytoextracts/active compounds may be considered as an important strategy for addressing the wound problem associated with DM in a better way.

CONCLUSION

Diabetes adversely acts on the phases of normal wound healing phases, i.e., hemostasis, inflammatory phase, proliferative phase, re-epithelialization and remodeling phase, and poses a big burden on the quality of life of a diabetic individual. Hyperglycemia can trigger oxidative stress, increase inflammatory cytokines, interrupt angiogenesis, decrease the functioning of fibroblast and keratinocyte, induce neuropathy associated events, increase MMPs, and reduce TIMPs that is responsible for impaired states of wound healing. An understanding sequence of the molecular and cellular cascade, epigenetic mechanisms, microbial perspective, complexity and plasticity of impaired wound healing in diabetic conditions is required for targeted research focusing on treatment of diabetic wound. Pharmacotherapy/strategy involving angiogenesis stimulation, growth factors, cytokines modulators, MMP inhibitors, ECM stimulators, anti-inflammatory drugs, antidiabetic agents, antimicrobial drugs, debridement, offloading, PRP, oxygen therapy, NPWT, LLLT, ESWT, stem cells, bio-engineered substitutes, and various natural-based products have shown their benefit. There has been a lack of quality-based evidence of efficacy of different adjuvant therapies tested through different clinical trials, thus more structured and quality studies are required. Indeed, the utilisation of medicinal plants/products in diabetic wound care holds prodigious potential in the future, and the development of innovative pharmaceutical formulations for advanced wound care is equally critical. Effective diabetic wound management necessitates a combination of techniques, including medication and non-pharmacological intervention. Hence, the treatment strategy of the future can only succeed if research concentrated on plausible drug targets after comprehending the inherent pathological complexities, evaluating non-pharmacological approaches through well-designed clinical trials, and targeting natural sources for new drug development.

FOOTNOTES

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REFERENCES

1 **Rodrigues M**, Kosaric N, Bonham CA, Gurtner GC. Wound Healing: A Cellular Perspective. *Physiol Rev* 2019; **99**: 665-706 [PMID: 30475656 DOI: 10.1152/physrev.00067.2017]
2 **Shah A**, Amini-Nik S. The Role of Phytochemicals in the Inflammatory Phase of Wound Healing. *Int J Mol Sci* 2017; **18** [PMID: 28509885 DOI: 10.3390/ijms18051068]
3 **Wilkinson HN**, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. *Open Biol* 2020; **10**: 200223 [PMID: 32993416 DOI: 10.1098/rsob.200223]
4 **Patel S**, Srivastava S, Singh MR, Singh D. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. *Biomed Pharmacother* 2019; **112**: 108615 [PMID: 30784919 DOI: 10.1016/j.biopha.2019.108615]
5 **Sen CK**. Human Wound and Its Burden: Updated 2020 Compendium of Estimates. *Adv Wound Care (New Rochelle)* 2021; **10**: 281-292 [PMID: 33733885 DOI: 10.1089/wound.2021.0026]
6 **Sun H**, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022; **183**: 109119 [PMID: 34879977 DOI: 10.1016/j.diabres.2021.109119]
7 **Ogurtsova K**, Guariguata L, Barengo NC, Ruiz PL, Sacre JW, Karuranga S, Sun H, Boyko EJ, Magliano DJ. IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. *Diabetes Res Clin Pract* 2022; **183**: 109118 [PMID: 34883189 DOI: 10.1016/j.diabres.2021.109118]
8 **Burgess JL**, Wyant WA, Abdo Abujamra B, Kirsner RS, Jozic I. Diabetic Wound-Healing Science. *Medicina (Kaunas)* 2021; **57** [PMID: 34684109 DOI: 10.3390/medicina57101072]
9 **Zhang P**, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis †. *Ann Med* 2017; **49**: 106-116 [PMID: 27585063 DOI: 10.1080/07853890.2016.1231932]
10 **Tsourdi E**, Barthel A, Rietzsch H, Reichel A, Bornstein SR. Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *Biomed Res Int* 2013; **2013**: 385641 [PMID: 23653894 DOI: 10.1155/2013/385641]
11 **Spampinato SF**, Caruso GI, De Pasquale R, Sortino MA, Merlo S. The Treatment of Impaired Wound Healing in Diabetes: Looking among Old Drugs. *Pharmaceuticals (Basel)* 2020; **13** [PMID: 32244718 DOI: 10.3390/ph13040060]
12 **Brem H**, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest* 2007; **117**: 1219-1222 [PMID: 17476353 DOI: 10.1172/JCI32169]
13 **Pastar I**, Ojeh N, Glinos GD, Stojadinovic O, Tomic-Canic M. Physiology and Pathophysiology of Wound Healing in Diabetes. In: The Diabetic Foot. 2018: 109-130 [DOI: 10.1007/978-3-319-89869-8_7]
14 **Maruyama K**, Asai J, Ii M, Thorne T, Losordo DW, D'Amore PA. Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. *Am J Pathol* 2007; **170**: 1178-1191 [PMID: 17392158 DOI: 10.2353/ajpath.2007.060018]
15 **Okizaki S**, Ito Y, Hosono K, Oba K, Ohkubo H, Amano H, Shichiri M, Majima M. Suppressed recruitment of alternatively activated macrophages reduces TGF-β1 and impairs wound healing in streptozotocin-induced diabetic mice. *Biomed Pharmacother* 2015; **70**: 317-325 [PMID: 25677561 DOI: 10.1016/j.biopha.2014.10.020]
16 **Falanga V**. Wound healing and its impairment in the diabetic foot. *Lancet* 2005; **366**: 1736-1743 [PMID: 16291068 DOI: 10.1016/S0140-6736(05)67700-8]
17 **LoGerfo FW**, Coffman JD. Current concepts. Vascular and microvascular disease of the foot in diabetes. Implications for foot care. *N Engl J Med* 1984; **311**: 1615-1619 [PMID: 6390204 DOI: 10.1056/NEJM198412203112506]
18 **Dinh T**, Veves A. Microcirculation of the diabetic foot. *Curr Pharm Des* 2005; **11**: 2301-2309 [PMID: 16022669 DOI: 10.2174/1381612054367328]
19 **Dinh TL**, Veves A. A review of the mechanisms implicated in the pathogenesis of the diabetic foot. *Int J Low Extrem Wounds* 2005; **4**: 154-159 [PMID: 16100096 DOI: 10.1177/1534734605280130]
20 **Lima AL**, Illing T, Schliemann S, Elsner P. Cutaneous Manifestations of Diabetes Mellitus: A Review. *Am J Clin Dermatol* 2017; **18**: 541-553 [PMID: 28374407 DOI: 10.1007/s40257-017-0275-z]
21 **Andrade TAM**, Masson-Meyers DS, Caetano GF, Terra VA, Ovidio PP, Jordão-Júnior AA, Frade MAC. Skin changes in streptozotocin-induced diabetic rats. *Biochem Biophys Res Commun* 2017; **490**: 1154-1161 [PMID: 28668393 DOI: 10.1016/j.bbrc.2017.06.166]
22 **Kim JH**, Yoon NY, Kim DH, Jung M, Jun M, Park HY, Chung CH, Lee K, Kim S, Park CS, Liu KH, Choi EH. Impaired permeability and antimicrobial barriers in type 2 diabetes skin are linked to increased serum levels of advanced glycation end-product. *Exp Dermatol* 2018; **27**: 815-823 [PMID: 29151267 DOI: 10.1111/exd.13466]
23 **Blakytny R**, Jude EB. Altered molecular mechanisms of diabetic foot ulcers. *Int J Low Extrem Wounds* 2009; **8**: 95-104 [PMID: 19443898 DOI: 10.1177/1534734609337151]
24 **Ryan MC**, Lee K, Miyashita Y, Carter WG. Targeted disruption of the LAMA3 gene in mice reveals abnormalities in

survival and late stage differentiation of epithelial cells. *J Cell Biol* 1999; **145**: 1309-1323 [PMID: 10366601 DOI: 10.1083/jcb.145.6.1309]

25 **Ramirez HA**, Liang L, Pastar I, Rosa AM, Stojadinovic O, Zwick TG, Kirsner RS, Maione AG, Garlick JA, Tomic-Canic M. Comparative Genomic, MicroRNA, and Tissue Analyses Reveal Subtle Differences between Non-Diabetic and Diabetic Foot Skin. *PLoS One* 2015; **10**: e0137133 [PMID: 26318001 DOI: 10.1371/journal.pone.0137133]

26 **Maione AG**, Smith A, Kashpur O, Yanez V, Knight E, Mooney DJ, Veves A, Tomic-Canic M, Garlick JA. Altered ECM deposition by diabetic foot ulcer-derived fibroblasts implicates fibronectin in chronic wound repair. *Wound Repair Regen* 2016; **24**: 630-643 [PMID: 27102877 DOI: 10.1111/wrr.12437]

27 **Liang L**, Stone RC, Stojadinovic O, Ramirez H, Pastar I, Maione AG, Smith A, Yanez V, Veves A, Kirsner RS, Garlick JA, Tomic-Canic M. Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. *Wound Repair Regen* 2016; **24**: 943-953 [PMID: 27607190 DOI: 10.1111/wrr.12470]

28 **Maione AG**, Brudno Y, Stojadinovic O, Park LK, Smith A, Tellechea A, Leal EC, Kearney CJ, Veves A, Tomic-Canic M, Mooney DJ, Garlick JA. Three-dimensional human tissue models that incorporate diabetic foot ulcer-derived fibroblasts mimic *in vivo* features of chronic wounds. *Tissue Eng Part C Methods* 2015; **21**: 499-508 [PMID: 25343343 DOI: 10.1089/ten.TEC.2014.0414]

29 **Berlanga-Acosta J**, Mendoza-Mari Y, Martínez MD, Valdés-Perez C, Ojalvo AG, Armstrong DG. Expression of cell proliferation cycle negative regulators in fibroblasts of an ischemic diabetic foot ulcer. A clinical case report. *Int Wound J* 2013; **10**: 232-236 [PMID: 23194110 DOI: 10.1111/j.1742-481X.2012.12000.x]

30 **Caballero S**, Sengupta N, Afzal A, Chang KH, Li Calzi S, Guberski DL, Kern TS, Grant MB. Ischemic vascular damage can be repaired by healthy, but not diabetic, endothelial progenitor cells. *Diabetes* 2007; **56**: 960-967 [PMID: 17395742 DOI: 10.2337/db06-1254]

31 **Liu ZJ**, Velazquez OC. Hyperoxia, endothelial progenitor cell mobilization, and diabetic wound healing. *Antioxid Redox Signal* 2008; **10**: 1869-1882 [PMID: 18627349 DOI: 10.1089/ars.2008.2121]

32 **Yu CG**, Zhang N, Yuan SS, Ma Y, Yang LY, Feng YM, Zhao D. Endothelial Progenitor Cells in Diabetic Microvascular Complications: Friends or Foes? *Stem Cells Int* 2016; **2016**: 1803989 [PMID: 27313624 DOI: 10.1155/2016/1803989]

33 **Mirza RE**, Fang MM, Novak ML, Urao N, Sui A, Ennis WJ, Koh TJ. Macrophage PPARγ and impaired wound healing in type 2 diabetes. *J Pathol* 2015; **236**: 433-444 [PMID: 25875529 DOI: 10.1002/path.4548]

34 **Krisp C**, Jacobsen F, McKay MJ, Molloy MP, Steinstraesser L, Wolters DA. Proteome analysis reveals antiangiogenic environments in chronic wounds of diabetes mellitus type 2 patients. *Proteomics* 2013; **13**: 2670-2681 [PMID: 23798543 DOI: 10.1002/pmic.201200502]

35 **Liu Y**, Min D, Bolton T, Nubé V, Twigg SM, Yue DK, McLennan SV. Increased matrix metalloproteinase-9 predicts poor wound healing in diabetic foot ulcers. *Diabetes Care* 2009; **32**: 117-119 [PMID: 18835949 DOI: 10.2337/dc08-0763]

36 **Lobmann R**, Ambrosch A, Schultz G, Waldmann K, Schiweck S, Lehnert H. Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. *Diabetologia* 2002; **45**: 1011-1016 [PMID: 12136400 DOI: 10.1007/s00125-002-0868-8]

37 **Obrosova IG**. Update on the pathogenesis of diabetic neuropathy. *Curr Diab Rep* 2003; **3**: 439-445 [PMID: 14611738 DOI: 10.1007/s11892-003-0005-1]

38 **Wang C**, Li C, Peng H, Ye Z, Zhang J, Liu X, Lou T. Activation of the Nrf2-ARE pathway attenuates hyperglycemia-mediated injuries in mouse podocytes. *Cell Physiol Biochem* 2014; **34**: 891-902 [PMID: 25200066 DOI: 10.1159/000366307]

39 **Lee YJ**, Kwon SB, An JM, Kim CH, Lee SH, Choi CY, Nam DH, Park JW, Nam HS, Lee MW, Cho MK. Increased protein oxidation and decreased expression of nuclear factor E2-related factor 2 protein in skin tissue of patients with diabetes. *Clin Exp Dermatol* 2015; **40**: 192-200 [PMID: 25557240 DOI: 10.1111/ced.12487]

40 **Long M**, Rojo de la Vega M, Wen Q, Bharara M, Jiang T, Zhang R, Zhou S, Wong PK, Wondrak GT, Zheng H, Zhang DD. An Essential Role of NRF2 in Diabetic Wound Healing. *Diabetes* 2016; **65**: 780-793 [PMID: 26718502 DOI: 10.2337/db15-0564]

41 **Fadini GP**, Menegazzo L, Rigato M, Scattolini V, Poncina N, Bruttocao A, Ciciliot S, Mammano F, Ciubotaru CD, Brocco E, Marescotti MC, Cappellari R, Arrigoni G, Millioni R, Vigili de Kreutzenberg S, Albiero M, Avogaro A. NETosis Delays Diabetic Wound Healing in Mice and Humans. *Diabetes* 2016; **65**: 1061-1071 [PMID: 26740598 DOI: 10.2337/db15-0863]

42 **Deng L**, Du C, Song P, Chen T, Rui S, Armstrong DG, Deng W. The Role of Oxidative Stress and Antioxidants in Diabetic Wound Healing. *Oxid Med Cell Longev* 2021; **2021**: 8852759 [PMID: 33628388 DOI: 10.1155/2021/8852759]

43 **Yamaoka H**, Sasaki H, Yamasaki H, Ogawa K, Ohta T, Furuta H, Nishi M, Nanjo K. Truncal pruritus of unknown origin may be a symptom of diabetic polyneuropathy. *Diabetes Care* 2010; **33**: 150-155 [PMID: 20040674 DOI: 10.2337/dc09-0632]

44 **Decker P**, Muller S. Modulating poly (ADP-ribose) polymerase activity: potential for the prevention and therapy of pathogenic situations involving DNA damage and oxidative stress. *Curr Pharm Biotechnol* 2002; **3**: 275-283 [PMID: 12164482 DOI: 10.2174/1389201023378265]

45 **Caporali A**, Meloni M, Völlenkle C, Bonci D, Sala-Newby GB, Addis R, Spinetti G, Losa S, Masson R, Baker AH, Agami R, le Sage C, Condorelli G, Madeddu P, Martelli F, Emanueli C. Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. *Circulation* 2011; **123**: 282-291 [PMID: 21220732 DOI: 10.1161/CIRCULATIONAHA.110.952325]

46 **Rafehi H**, El-Osta A, Karagiannis TC. Epigenetic mechanisms in the pathogenesis of diabetic foot ulcers. *J Diabetes Complications* 2012; **26**: 554-561 [PMID: 22739801 DOI: 10.1016/j.jdiacomp.2012.05.015]

47 **Madhyastha R**, Madhyastha H, Nakajima Y, Omura S, Maruyama M. MicroRNA signature in diabetic wound healing: promotive role of miR-21 in fibroblast migration. *Int Wound J* 2012; **9**: 355-361 [PMID: 22067035 DOI: 10.1111/j.1742-481X.2011.00890.x]

48 **Wang X**, Cao Q, Yu L, Shi H, Xue B. Epigenetic regulation of macrophage polarization and inflammation by DNA methylation in obesity. *JCI Insight* 2016; **1**: e87748 [PMID: 27882346 DOI: 10.1172/jci.insight.87748]

49 **Yan J**, Tie G, Wang S, Tutto A, DeMarco N, Khair L, Fazzio TG, Messina LM. Diabetes impairs wound healing by Dnmt1-dependent dysregulation of hematopoietic stem cells differentiation towards macrophages. *Nat Commun* 2018; **9**: 33 [PMID: 29295997 DOI: 10.1038/s41467-017-02425-z]

50 **Ling L**, Ren M, Yang C, Lao G, Chen L, Luo H, Feng Z, Yan L. Role of site-specific DNA demethylation in TNFα-induced MMP9 expression in keratinocytes. *J Mol Endocrinol* 2013; **50**: 279-290 [PMID: 23417766 DOI: 10.1530/JME-12-0172]

51 **Zhang J**, Yang C, Wang C, Liu D, Lao G, Liang Y, Sun K, Luo H, Tan Q, Ren M, Yan L. AGE-induced keratinocyte MMP-9 expression is linked to TET2-mediated CpG demethylation. *Wound Repair Regen* 2016; **24**: 489-500 [PMID: 26913994 DOI: 10.1111/wrr.12426]

52 **Dhawan S**, Tschen SI, Zeng C, Guo T, Hebrok M, Matveyenko A, Bhushan A. DNA methylation directs functional maturation of pancreatic β cells. *J Clin Invest* 2015; **125**: 2851-2860 [PMID: 26098213 DOI: 10.1172/JCI79956]

53 **Chen Z**, Miao F, Paterson AD, Lachin JM, Zhang L, Schones DE, Wu X, Wang J, Tompkins JD, Genuth S, Braffett BH, Riggs AD; DCCT/EDIC Research Group, Natarajan R. Epigenomic profiling reveals an association between persistence of DNA methylation and metabolic memory in the DCCT/EDIC type 1 diabetes cohort. *Proc Natl Acad Sci U S A* 2016; **113**: E3002-E3011 [PMID: 27162351 DOI: 10.1073/pnas.1603712113]

54 **Robert I**, Aussems M, Keutgens A, Zhang X, Hennuy B, Viatour P, Vanstraelen G, Merville MP, Chapelle JP, de Leval L, Lambert F, Dejardin E, Gothot A, Chariot A. Matrix Metalloproteinase-9 gene induction by a truncated oncogenic NF-kappaB2 protein involves the recruitment of MLL1 and MLL2 H3K4 histone methyltransferase complexes. *Oncogene* 2009; **28**: 1626-1638 [PMID: 19219072 DOI: 10.1038/onc.2009.6]

55 **Carson WF 4th**, Cavassani KA, Soares EM, Hirai S, Kittan NA, Schaller MA, Scola MM, Joshi A, Matsukawa A, Aronoff DM, Johnson CN, Dou Y, Gallagher KA, Kunkel SL. The STAT4/MLL1 Epigenetic Axis Regulates the Antimicrobial Functions of Murine Macrophages. *J Immunol* 2017; **199**: 1865-1874 [PMID: 28733487 DOI: 10.4049/jimmunol.1601272]

56 **Wang X**, Zhu K, Li S, Liao Y, Du R, Zhang X, Shu HB, Guo AY, Li L, Wu M. MLL1, a H3K4 methyltransferase, regulates the TNFα-stimulated activation of genes downstream of NF-κB. *J Cell Sci* 2012; **125**: 4058-4066 [PMID: 22623725 DOI: 10.1242/jcs.103531]

57 **Kimball AS**, Joshi A, Carson WF 4th, Boniakowski AE, Schaller M, Allen R, Bermick J, Davis FM, Henke PK, Burant CF, Kunkel SL, Gallagher KA. The Histone Methyltransferase MLL1 Directs Macrophage-Mediated Inflammation in Wound Healing and Is Altered in a Murine Model of Obesity and Type 2 Diabetes. *Diabetes* 2017; **66**: 2459-2471 [PMID: 28663191 DOI: 10.2337/db17-0194]

58 **Gallagher KA**, Joshi A, Carson WF, Schaller M, Allen R, Mukerjee S, Kittan N, Feldman EL, Henke PK, Hogaboam C, Burant CF, Kunkel SL. Epigenetic changes in bone marrow progenitor cells influence the inflammatory phenotype and alter wound healing in type 2 diabetes. *Diabetes* 2015; **64**: 1420-1430 [PMID: 25368099 DOI: 10.2337/db14-0872]

59 **De Santa F**, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G. The histone H3 Lysine-27 demethylase Jmjd3 Links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* 2007; **130**: 1083-1094 [PMID: 17825402 DOI: 10.1016/j.cell.2007.08.019]

60 **Zimmermann M**, Aguilera FB, Castellucci M, Rossato M, Costa S, Lunardi C, Ostuni R, Girolomoni G, Natoli G, Bazzoni F, Tamassia N, Cassatella MA. Chromatin remodelling and autocrine TNFα are required for optimal interleukin-6 expression in activated human neutrophils. *Nat Commun* 2015; **6**: 6061 [PMID: 25616107 DOI: 10.1038/ncomms7061]

61 **Na J**, Shin JY, Jeong H, Lee JY, Kim BJ, Kim WS, Yune TY, Ju BG. JMJD3 and NF-κB-dependent activation of Notch1 gene is required for keratinocyte migration during skin wound healing. *Sci Rep* 2017; **7**: 6494 [PMID: 28747631 DOI: 10.1038/s41598-017-06750-7]

62 **den Dekker A**, Davis FM, Kunkel SL, Gallagher KA. Targeting epigenetic mechanisms in diabetic wound healing. *Transl Res* 2019; **204**: 39-50 [PMID: 30392877 DOI: 10.1016/j.trsl.2018.10.001]

63 **Thimmappaiah Jagadeesh A**, Prakash PY, Karthik Rao N, Ramya V. Culture characterization of the skin microbiome in Type 2 diabetes mellitus: A focus on the role of innate immunity. *Diabetes Res Clin Pract* 2017; **134**: 1-7 [PMID: 28951341 DOI: 10.1016/j.diabres.2017.09.007]

64 **Dörr S**, Holland-Letz AK, Weisser G, Chatzitomaris A, Lobmann R. Bacterial Diversity, Antibiotic Resistance, and the Risk of Lower Limb Amputation in Younger and Older Individuals With Diabetic Foot Infection. *Int J Low Extrem Wounds* 2021; 1534734621992290 [PMID: 33745353 DOI: 10.1177/1534734621992290]

65 **Radzieta M**, Sadeghpour-Heravi F, Peters TJ, Hu H, Vickery K, Jeffries T, Dickson HG, Schwarzer S, Jensen SO, Malone M. A multiomics approach to identify host-microbe alterations associated with infection severity in diabetic foot infections: a pilot study. *NPJ Biofilms Microbiomes* 2021; **7**: 29 [PMID: 33753735 DOI: 10.1038/s41522-021-00202-x]

66 **Gardner SE**, Hillis SL, Heilmann K, Segre JA, Grice EA. The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. *Diabetes* 2013; **62**: 923-930 [PMID: 23139351 DOI: 10.2337/db12-0771]

67 **van Asten SA**, La Fontaine J, Peters EJ, Bhavan K, Kim PJ, Lavery LA. The microbiome of diabetic foot osteomyelitis. *Eur J Clin Microbiol Infect Dis* 2016; **35**: 293-298 [PMID: 26670675 DOI: 10.1007/s10096-015-2544-1]

68 **Ezeani IU**, Ugwu ET, Adeleye FO, Gezawa ID, Okpe IO, Enamino MI. Determinants of wound healing in patients hospitalized for diabetic foot ulcer: results from the MEDFUN study. *Endocr Regul* 2020; **54**: 207-216 [PMID: 32857716 DOI: 10.2478/enr-2020-0023]

69 **Sumitomo T**, Mori Y, Nakamura Y, Honda-Ogawa M, Nakagawa S, Yamaguchi M, Matsue H, Terao Y, Nakata M, Kawabata S. Streptococcal Cysteine Protease-Mediated Cleavage of Desmogleins Is Involved in the Pathogenesis of Cutaneous Infection. *Front Cell Infect Microbiol* 2018; **8**: 10 [PMID: 29416987 DOI: 10.3389/fcimb.2018.00010]

70 **Greener B**, Hughes AA, Bannister NP, Douglass J. Proteases and pH in chronic wounds. *J Wound Care* 2005; **14**: 59-61 [PMID: 15739652 DOI: 10.12968/jowc.2005.14.2.26739]

71 **McArdle CD**, Lagan KM, McDowell DA. Effects of pH on the Antibiotic Resistance of Bacteria Recovered from Diabetic Foot Ulcer Fluid An In Vitro Study. *J Am Podiatr Med Assoc* 2018; **108**: 6-11 [PMID: 29547034 DOI:

10.7547/16-033]
72 **Armstrong DG**, Lavery LA. Diabetic foot ulcers: prevention, diagnosis and classification. *Am Fam Physician* 1998; **57**: 1325-1332, 1337 [PMID: 9531915]
73 **Bowering CK**. Diabetic foot ulcers. Pathophysiology, assessment, and therapy. *Can Fam Physician* 2001; **47**: 1007-1016 [PMID: 11398715]
74 **Frykberg RG**. Diabetic foot ulcers: pathogenesis and management. *Am Fam Physician* 2002; **66**: 1655-1662 [PMID: 12449264]
75 **Lavery LA**, Peters EJ, Williams JR, Murdoch DP, Hudson A, Lavery DC; International Working Group on the Diabetic Foot. Reevaluating the way we classify the diabetic foot: restructuring the diabetic foot risk classification system of the International Working Group on the Diabetic Foot. *Diabetes Care* 2008; **31**: 154-156 [PMID: 17934155 DOI: 10.2337/dc07-1302]
76 **Schaper NC**, Nabuurs-Franssen MH. The diabetic foot: pathogenesis and clinical evaluation. *Semin Vasc Med* 2002; **2**: 221-228 [PMID: 16222613 DOI: 10.1055/s-2002-32045]
77 **Searles JM Jr**, Colen LB. Foot reconstruction in diabetes mellitus and peripheral vascular insufficiency. *Clin Plast Surg* 1991; **18**: 467-483 [PMID: 1889157]
78 **Boulton AJ**, Kirsner RS, Vileikyte L. Clinical practice. Neuropathic diabetic foot ulcers. *N Engl J Med* 2004; **351**: 48-55 [PMID: 15229307 DOI: 10.1056/NEJMcp032966]
79 **Chand G**, Mishra AK, Kumar S, Agarwal A. Diabetic foot. *Clinl Queries Nephrol* 2012; **2**: 144-150
80 **Vallianou N**, Evangelopoulos A, Koutalas P. Alpha-lipoic Acid and diabetic neuropathy. *Rev Diabet Stud* 2009; **6**: 230-236 [PMID: 20043035 DOI: 10.1900/RDS.2009.6.230]
81 **Benrath J**, Zimmermann M, Gillardon F. Substance P and nitric oxide mediate would healing of ultraviolet photodamaged rat skin: evidence for an effect of nitric oxide on keratinocyte proliferation. *Neurosci Lett* 1995; **200**: 17-20 [PMID: 8584255 DOI: 10.1016/0304-3940(95)12062-9]
82 **Ghersi G**, Chen W, Lee EW, Zukowska Z. Critical role of dipeptidyl peptidase IV in neuropeptide Y-mediated endothelial cell migration in response to wounding. *Peptides* 2001; **22**: 453-458 [PMID: 11287101 DOI: 10.1016/s0196-9781(01)00340-0]
83 **Peleg AY**, Weerarathna T, McCarthy JS, Davis TM. Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. *Diabetes Metab Res Rev* 2007; **23**: 3-13 [PMID: 16960917 DOI: 10.1002/dmrr.682]
84 **Smith K**, Collier A, Townsend EM, O'Donnell LE, Bal AM, Butcher J, Mackay WG, Ramage G, Williams C. One step closer to understanding the role of bacteria in diabetic foot ulcers: characterising the microbiome of ulcers. *BMC Microbiol* 2016; **16**: 54 [PMID: 27005417 DOI: 10.1186/s12866-016-0665-z]
85 **Basu Mallik S**, Jayashree BS, Shenoy RR. Epigenetic modulation of macrophage polarization- perspectives in diabetic wounds. *J Diabetes Complications* 2018; **32**: 524-530 [PMID: 29530315 DOI: 10.1016/j.jdiacomp.2018.01.015]
86 **Kwon KT**, Armstrong DG. Microbiology and Antimicrobial Therapy for Diabetic Foot Infections. *Infect Chemother* 2018; **50**: 11-20 [PMID: 29637748 DOI: 10.3947/ic.2018.50.1.11]
87 **Brownlee M**. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615-1625 [PMID: 15919781 DOI: 10.2337/diabetes.54.6.1615]
88 **Alavi A**, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, Woo K, Boeni T, Ayello EA, Kirsner RS. Diabetic foot ulcers: Part I. Pathophysiology and prevention. *J Am Acad Dermatol* 2014; **70**: 1.e1-18; quiz 19 [PMID: 24355275 DOI: 10.1016/j.jaad.2013.06.055]
89 **Tsalamandris S**, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, Deftereos S, Tousoulis D. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *Eur Cardiol* 2019; **14**: 50-59 [PMID: 31131037 DOI: 10.15420/ecr.2018.33.1]
90 **Loot MA**, Kenter SB, Au FL, van Galen WJ, Middelkoop E, Bos JD, Mekkes JR. Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls. *Eur J Cell Biol* 2002; **81**: 153-160 [PMID: 11998867 DOI: 10.1078/0171-9335-00228]
91 **Nishikori Y**, Shiota N, Okunishi H. The role of mast cells in cutaneous wound healing in streptozotocin-induced diabetic mice. *Arch Dermatol Res* 2014; **306**: 823-835 [PMID: 25218083 DOI: 10.1007/s00403-014-1496-0]
92 **Tellechea A**, Leal EC, Kafanas A, Auster ME, Kuchibhotla S, Ostrovsky Y, Tecilazich F, Baltzis D, Zheng Y, Carvalho E, Zabolotny JM, Weng Z, Petra A, Patel A, Panagiotidou S, Pradhan-Nabzdyk L, Theoharides TC, Veves A. Mast Cells Regulate Wound Healing in Diabetes. *Diabetes* 2016; **65**: 2006-2019 [PMID: 27207516 DOI: 10.2337/db15-0340]
93 **Bevan D**, Gherardi E, Fan TP, Edwards D, Warn R. Diverse and potent activities of HGF/SF in skin wound repair. *J Pathol* 2004; **203**: 831-838 [PMID: 15221943 DOI: 10.1002/path.1578]
94 **Singh K**, Agrawal NK, Gupta SK, Mohan G, Chaturvedi S, Singh K. Decreased expression of heat shock proteins may lead to compromised wound healing in type 2 diabetes mellitus patients. *J Diabetes Complications* 2015; **29**: 578-588 [PMID: 25746357 DOI: 10.1016/j.jdiacomp.2015.01.007]
95 **Luong M**, Zhang Y, Chamberlain T, Zhou T, Wright JF, Dower K, Hall JP. Stimulation of TLR4 by recombinant HSP70 requires structural integrity of the HSP70 protein itself. *J Inflamm (Lond)* 2012; **9**: 11 [PMID: 22448747 DOI: 10.1186/1476-9255-9-11]
96 **Park KH**, Han SH, Hong JP, Han SK, Lee DH, Kim BS, Ahn JH, Lee JW. Topical epidermal growth factor spray for the treatment of chronic diabetic foot ulcers: A phase III multicenter, double-blind, randomized, placebo-controlled trial. *Diabetes Res Clin Pract* 2018; **142**: 335-344 [PMID: 29902542 DOI: 10.1016/j.diabres.2018.06.002]
97 **Barrientos S**, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. *Wound Repair Regen* 2008; **16**: 585-601 [PMID: 19128254 DOI: 10.1111/j.1524-475X.2008.00410.x]
98 **Greenhalgh DG**, Sprugel KH, Murray MJ, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *Am J Pathol* 1990; **136**: 1235-1246 [PMID: 2356856]
99 **Doxey DL**, Ng MC, Dill RE, Iacopino AM. Platelet-derived growth factor levels in wounds of diabetic rats. *Life Sci* 1995; **57**: 1111-1123 [PMID: 7658918 DOI: 10.1016/0024-3205(95)02056-o]

100 **Li H**, Fu X, Zhang L, Huang Q, Wu Z, Sun T. Research of PDGF-BB gel on the wound healing of diabetic rats and its pharmacodynamics. *J Surg Res* 2008; **145**: 41-48 [PMID: 18082770 DOI: 10.1016/j.jss.2007.02.044]

101 **Lobmann R**, Zemlin C, Motzkau M, Reschke K, Lehnert H. Expression of matrix metalloproteinases and growth factors in diabetic foot wounds treated with a protease absorbent dressing. *J Diabetes Complications* 2006; **20**: 329-335 [PMID: 16949521 DOI: 10.1016/j.jdiacomp.2005.08.007]

102 **Curran MP**, Plosker GL. Bilayered bioengineered skin substitute (Apligraf): a review of its use in the treatment of venous leg ulcers and diabetic foot ulcers. *BioDrugs* 2002; **16**: 439-455 [PMID: 12463767 DOI: 10.2165/00063030-200216060-00005]

103 **Tsuboi R**, Rifkin DB. Recombinant basic fibroblast growth factor stimulates wound healing in healing-impaired db/db mice. *J Exp Med* 1990; **172**: 245-251 [PMID: 2358777 DOI: 10.1084/jem.172.1.245]

104 **Connolly DT**, Stoddard BL, Harakas NK, Feder J. Human fibroblast-derived growth factor is a mitogen and chemoattractant for endothelial cells. *Biochem Biophys Res Commun* 1987; **144**: 705-712 [PMID: 3579937 DOI: 10.1016/s0006-291x(87)80022-0]

105 **Kurokawa M**, Nakamura H, inventors; Polypeptide with epidermal regeneration accelerating minimal amino acid sequences, a polyalkylenepolyamine and/or polyarylenepolyamine, and a sheet. United States patent 7576051. 2009 August 18.

106 **Presta LG**, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, Winkler M, Ferrara N. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res* 1997; **57**: 4593-4599 [PMID: 9377574]

107 **Zhou K**, Ma Y, Brogan MS. Chronic and non-healing wounds: The story of vascular endothelial growth factor. *Med Hypotheses* 2015; **85**: 399-404 [PMID: 26138626 DOI: 10.1016/j.mehy.2015.06.017]

108 **Angelo LS**, Kurzrock R. Vascular endothelial growth factor and its relationship to inflammatory mediators. *Clin Cancer Res* 2007; **13**: 2825-2830 [PMID: 17504979 DOI: 10.1158/1078-0432.CCR-06-2416]

109 **Hardwicke JT**, Hart J, Bell A, Duncan R, Thomas DW, Moseley R. The effect of dextrin-rhEGF on the healing of full-thickness, excisional wounds in the (db/db) diabetic mouse. *J Control Release* 2011; **152**: 411-417 [PMID: 21435363 DOI: 10.1016/j.jconrel.2011.03.016]

110 **Brown DL**, Kane CD, Chernausek SD, Greenhalgh DG. Differential expression and localization of insulin-like growth factors I and II in cutaneous wounds of diabetic and nondiabetic mice. *Am J Pathol* 1997; **151**: 715-724 [PMID: 9284820]

111 **Bruhn-Olszewska B**, Korzon-Burakowska A, Gabig-Cimińska M, Olszewski P, Węgrzyn A, Jakóbkiewicz-Banecka J. Molecular factors involved in the development of diabetic foot syndrome. *Acta Biochim Pol* 2012; **59**: 507-513 [PMID: 23251910]

112 **Bhora FY**, Dunkin BJ, Batzri S, Aly HM, Bass BL, Sidawy AN, Harmon JW. Effect of growth factors on cell proliferation and epithelialization in human skin. *J Surg Res* 1995; **59**: 236-244 [PMID: 7543631 DOI: 10.1006/jsre.1995.1160]

113 **Armstrong DG**, Jude EB. The role of matrix metalloproteinases in wound healing. *J Am Podiatr Med Assoc* 2002; **92**: 12-18 [PMID: 11796794 DOI: 10.7547/87507315-92-1-12]

114 **Lobmann R**, Pap T, Ambrosch A, Waldmann K, König W, Lehnert H. Differential effects of PDGF-BB on matrix metalloproteases and cytokine release in fibroblasts of Type 2 diabetic patients and normal controls in vitro. *J Diabetes Complications* 2006; **20**: 105-112 [PMID: 16504839 DOI: 10.1016/j.jdiacomp.2005.05.013]

115 **Gary Sibbald R**, Woo KY. The biology of chronic foot ulcers in persons with diabetes. *Diabetes Metab Res Rev* 2008; **24** Suppl 1: S25-S30 [PMID: 18442179 DOI: 10.1002/dmrr.847]

116 **Dash NR**, Dash SN, Routray P, Mohapatra S, Mohapatra PC. Targeting nonhealing ulcers of lower extremity in human through autologous bone marrow-derived mesenchymal stem cells. *Rejuvenation Res* 2009; **12**: 359-366 [PMID: 19929258 DOI: 10.1089/rej.2009.0872]

117 **Yang M**, Sheng L, Zhang TR, Li Q. Stem cell therapy for lower extremity diabetic ulcers: where do we stand? *Biomed Res Int* 2013; **2013**: 462179 [PMID: 23586040 DOI: 10.1155/2013/462179]

118 **Chong HC**, Chan JS, Goh CQ, Gounko NV, Luo B, Wang X, Foo S, Wong MT, Choong C, Kersten S, Tan NS. Angiopoietin-like 4 stimulates STAT3-mediated iNOS expression and enhances angiogenesis to accelerate wound healing in diabetic mice. *Mol Ther* 2014; **22**: 1593-1604 [PMID: 24903577 DOI: 10.1038/mt.2014.102]

119 **Laing T**, Hanson R, Chan F, Bouchier-Hayes D. Effect of pravastatin on experimental diabetic wound healing. *J Surg Res* 2010; **161**: 336-340 [PMID: 20031169 DOI: 10.1016/j.jss.2009.01.024]

120 **Bagheri M**, Jahromi BM, Mirkhani H, Solhjou Z, Noorafshan A, Zamani A, Amirghofran Z. Azelnidipine, a new calcium channel blocker, promotes skin wound healing in diabetic rats. *J Surg Res* 2011; **169**: e101-e107 [PMID: 21571319 DOI: 10.1016/j.jss.2011.02.039]

121 **Leu JG**, Chiang MH, Chen CY, Lin JT, Chen HM, Chen YL, Liang YJ. Adenine accelerated the diabetic wound healing by PPAR delta and angiogenic regulation. *Eur J Pharmacol* 2018; **818**: 569-577 [PMID: 29162431 DOI: 10.1016/j.ejphar.2017.11.027]

122 **Lee Y**, Chang JJ, Yang MC, Chien CT, Lai, WF Acceleration of wound healing in diabetic rats by layered hydrogel dressing.
Carbohydr Polym 2021; 88: 809–819 [DOI: 10.1016/j.carbpol.2011.12.045]

123 Aly UF. Preparation and evaluation of novel topical gel preparations for wound healing in diabetics. *Int J Pharm Pharm Sci* 2012; **4**: 76-77

124 **Moon KC**, Lee JS, Han SK, Lee HW, Dhong ES. Effects of human umbilical cord blood-derived mesenchymal stromal cells and dermal fibroblasts on diabetic wound healing. *Cytotherapy* 2017; **19**: 821-828 [PMID: 28462822 DOI: 10.1016/j.jcyt.2017.03.074]

125 **Dixon D**, Edmonds M. Managing Diabetic Foot Ulcers: Pharmacotherapy for Wound Healing. *Drugs* 2021; **81**: 29-56 [PMID: 33382445 DOI: 10.1007/s40265-020-01415-8]

126 **Ramirez-Acuña JM**, Cardenas-Cadena SA, Marquez-Salas PA, Garza-Veloz I, Perez-Favila A, Cid-Baez MA, Flores-Morales V, Martinez-Fierro ML. Diabetic Foot Ulcers: Current Advances in Antimicrobial Therapies and Emerging

Treatments. *Antibiotics (Basel)* 2019; **8** [PMID: 31652990 DOI: 10.3390/antibiotics8040193]
127 **Kavitha KV**, Tiwari S, Purandare VB, Khedkar S, Bhosale SS, Unnikrishnan AG. Choice of wound care in diabetic foot ulcer: A practical approach. *World J Diabetes* 2014; **5**: 546-556 [PMID: 25126400 DOI: 10.4239/wjd.v5.i4.546]
128 **Tecilazich F**, Dinh TL, Veves A. Emerging drugs for the treatment of diabetic ulcers. *Expert Opin Emerg Drugs* 2013; **18**: 207-217 [PMID: 23687931 DOI: 10.1517/14728214.2013.802305]
129 **Karri VV**, Kuppusamy G, Talluri SV, Yamjala K, Mannemala SS, Malayandi R. Current and emerging therapies in the management of diabetic foot ulcers. *Curr Med Res Opin* 2016; **32**: 519-542 [PMID: 26643047 DOI: 10.1185/03007995.2015.1128888]
130 **Hingorani A**, LaMuraglia GM, Henke P, Meissner MH, Loretz L, Zinszer KM, Driver VR, Frykberg R, Carman TL, Marston W, Mills JL Sr, Murad MH. The management of diabetic foot: A clinical practice guideline by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine. *J Vasc Surg* 2016; **63**: 3S-21S [PMID: 26804367 DOI: 10.1016/j.jvs.2015.10.003]
131 **Everett E**, Mathioudakis N. Update on management of diabetic foot ulcers. *Ann N Y Acad Sci* 2018; **1411**: 153-165 [PMID: 29377202 DOI: 10.1111/nyas.13569]
132 **Nayak C**, Singh V, Singh K, Singh H, Gupta J, Ali MS, Ponnam HB. A prospective observational study to ascertain the role of homeopathic therapy in the management of diabetic foot ulcer. *Indian J Res Homoeopathy* 2012; **6**: 22-31
133 **Ponnam HB**, Gupta J. Role of silicea in diabetic foot ulcer: A retrospectively analysed case series. *Homœopathic Links* 2020; **33**: 53-58 [DOI: 10.1055/s-0040-1705143]
134 **Huang YY**, Lin CW, Cheng NC, Cazzell SM, Chen HH, Huang KF, Tung KY, Huang HL, Lin PY, Perng CK, Shi B, Liu C, Ma Y, Cao Y, Li Y, Xue Y, Yan L, Li Q, Ning G, Chang SC. Effect of a Novel Macrophage-Regulating Drug on Wound Healing in Patients With Diabetic Foot Ulcers: A Randomized Clinical Trial. *JAMA Netw Open* 2021; **4**: e2122607 [PMID: 34477854 DOI: 10.1001/jamanetworkopen.2021.22607]
135 **Bagher ALM**, Ramin H, Bahrami A, Delshad H, Ranjbar Omrani G, Mohammad K, Heydarpour R, Mohajeri Tehrani MR, Kamali K, Farhadi M, Gharib Doust F, Madani SH. Effects of intravenous semelil (angiparstm) on diabetic foot ulcers healing: A multicenter clinical trial. *DARU J Pharma Sci* 2008; **16**: 35-40
136 **Nasiri M**, Fayazi S, Jahani S, Yazdanpanah L, Haghighizadeh MH. The effect of topical olive oil on the healing of foot ulcer in patients with type 2 diabetes: a double-blind randomized clinical trial study in Iran. *J Diabetes Metab Disord* 2015; **14**: 38 [PMID: 25969821 DOI: 10.1186/s40200-015-0167-9]
137 **Viswanathan V**, Kesavan R, Kavitha KV, Kumpatla S. A pilot study on the effects of a polyherbal formulation cream on diabetic foot ulcers. *Indian J Med Res* 2011; **134**: 168-173 [PMID: 21911968]
138 **Masoompour SM**, Bagheri MH, Borhani Haghighi A, Novitsky YA, Sadeghi B, Gharibdoust F, Larijani B, Ranjbar Omrani G. Effect of ANGIPARS™, a new herbal drug on diabetic foot ulcer: A phase 2 clinical study. *DARU* 2008; **16**: 31-34
139 **Mohammadi MH**, Molavi B, Mohammadi S, Nikbakht M, Mohammadi AM, Mostafaei S, Norooznezhad AH, Ghorbani Abdegah A, Ghavamzadeh A. Evaluation of wound healing in diabetic foot ulcer using platelet-rich plasma gel: A single-arm clinical trial. *Transfus Apher Sci* 2017; **56**: 160-164 [PMID: 27839965 DOI: 10.1016/j.transci.2016.10.020]
140 **Prakasam N**, Prabakar MS, Reshma S, Loganathan K, Senguttuvan K. A clinical study of platelet rich plasma *vs* conventional dressing in management of diabetic foot ulcers. *Int Surg J* 2018; **5**: 3210-3216 [DOI: 10.18203/2349-2902.isj20184069]
141 **Hong JP**, Jung HD, Kim YW. Recombinant human epidermal growth factor (EGF) to enhance healing for diabetic foot ulcers. *Ann Plast Surg* 2006; **56**: 394-8; discussion 399 [PMID: 16557070 DOI: 10.1097/01.sap.0000198731.12407.0c]
142 **Viswanathan V**, Juttada U, Babu M. Efficacy of Recombinant Human Epidermal Growth Factor (Regen-D 150) in Healing Diabetic Foot Ulcers: A Hospital-Based Randomized Controlled Trial. *Int J Low Extrem Wounds* 2020; **19**: 158-164 [PMID: 31878810 DOI: 10.1177/1534734619892791]
143 **Rangaswamy P**, Rubby SA, Prasanth K. Prospective study of platelet derived growth factor in wound healing of diabetic foot ulcers in Indian population. *Int Surg J* 2017; **4**: 194-199 [DOI: 10.18203/2349-2902.isj20164428]
144 **Samuel S**, Mahajan A, Mam MK, Prakash JS. Platelet derived growth factor in diabetic lower extremity ulcer: A randomized, double blind, placebo controlled study in Indian condition. *Int J Pharm Sci Res* 2016; **7**: 3887-3892 [DOI: 10.13040/IJPSR.0975-8232.7(9).3887-92]
145 **UCHI H**, IGARASHI A, URABE K, KOGA T, NAKAYAMA J, KAWAMORI R, TAMAKI K, HIRAKATA H, OHURA T, FURUE M. Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer. *Eur J Dermatol* 2009; **19**: 461-468 [PMID: 19638336 DOI: 10.1684/ejd.2009.0750]
146 **Serena TE**, Bullock NM, Cole W, Lantis J, Li L, Moore S, Patel K, Sabo M, Wahab N, Price P. Topical oxygen therapy in the treatment of diabetic foot ulcers: a multicentre, open, randomised controlled clinical trial. *J Wound Care* 2021; **30**: S7-S14 [PMID: 33979229 DOI: 10.12968/jowc.2021.30.Sup5.S7]
147 **Löndahl M**, Katzman P, Nilsson A, Hammarlund C. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. *Diabetes Care* 2010; **33**: 998-1003 [PMID: 20427683 DOI: 10.2337/dc09-1754]
148 **Maranna H**, Lal P, Mishra A, Bains L, Sawant G, Bhatia R, Kumar P, Beg MY. Negative pressure wound therapy in grade 1 and 2 diabetic foot ulcers: A randomized controlled study. *Diabetes Metab Syndr* 2021; **15**: 365-371 [PMID: 33524646 DOI: 10.1016/j.dsx.2021.01.014]
149 **Alamdari NM**, Mehraneroodi B, Gholizadeh B, Zeinalpour A, Safe P, Besharat S. The efficacy of negative pressure wound therapy compared with conventional dressing in treating infected diabetic foot ulcers: a randomized controlled trial. *Int J Diabetes Dev Ctries* 2021; **41**: 664-668 [DOI: 10.1007/s13410-021-00941-9]
150 **Kaviani A**, Djavid GE, Ataie-Fashtami L, Fateh M, Ghodsi M, Salami M, Zand N, Kashef N, Larijani B. A randomized clinical trial on the effect of low-level laser therapy on chronic diabetic foot wound healing: a preliminary report. *Photomed Laser Surg* 2011; **29**: 109-114 [PMID: 21214368 DOI: 10.1089/pho.2009.2680]
151 **Salvi M**, Rimini D, Molinari F, Bestente G, Alberto Bruno M. D. Effect of low-level light therapy on diabetic foot ulcers: a near-infrared spectroscopy study. *J Biomed Optics* 2017; **22**: 038001 [DOI: 10.1117/1.JBO.22.3.038001]

152 **Omar MT**, Alghadir A, Al-Wahhabi KK, Al-Askar AB. Efficacy of shock wave therapy on chronic diabetic foot ulcer: a single-blinded randomized controlled clinical trial. *Diabetes Res Clin Pract* 2014; **106**: 548-554 [PMID: 25451894 DOI: 10.1016/j.diabres.2014.09.024]

153 **Jeppesen SM**, Yderstraede KB, Rasmussen BS, Hanna M, Lund L. Extracorporeal shockwave therapy in the treatment of chronic diabetic foot ulcers: a prospective randomised trial. *J Wound Care* 2016; **25**: 641-649 [PMID: 27827284 DOI: 10.12968/jowc.2016.25.11.641]

154 **Maksimova N**, Krasheninnikov M, Zhang Y, Ponomarev E, Pomytkin I, Melnichenko G, Lyundup A. Early passage autologous mesenchymal stromal cells accelerate diabetic wound re-epithelialization: A clinical case study. *Cytotherapy* 2017; **19**: 1548-1550 [PMID: 28986173 DOI: 10.1016/j.jcyt.2017.08.017]

155 **Qin HL**, Zhu XH, Zhang B, Zhou L, Wang WY. Clinical Evaluation of Human Umbilical Cord Mesenchymal Stem Cell Transplantation After Angioplasty for Diabetic Foot. *Exp Clin Endocrinol Diabetes* 2016; **124**: 497-503 [PMID: 27219884 DOI: 10.1055/s-0042-103684]

156 **Edmonds ME**, Bodansky HJ, Boulton AJM, Chadwick PJ, Dang CN, D'Costa R, Johnston A, Kennon B, Leese G, Rajbhandari SM, Serena TE, Young MJ, Stewart JE, Tucker AT, Carter MJ. Multicenter, randomized controlled, observer-blinded study of a nitric oxide generating treatment in foot ulcers of patients with diabetes-ProNOx1 study. Wound Repair Regen 2018; 26: 228-237 [PMID: 29617058 DOI: 10.1111/wrr.12630]

157 **Rullan M**, Cerdà L, Frontera G, Masmiquel L, Llobera J. Treatment of chronic diabetic foot ulcers with bemiparin: a randomized, triple-blind, placebo-controlled, clinical trial. *Diabet Med* 2008; **25**: 1090-1095 [PMID: 19183313 DOI: 10.1111/j.1464-5491.2008.02527.x]

158 **Imran M**, Hussain MB, Baig M. A Randomized, Controlled Clinical Trial of Honey-Impregnated Dressing for Treating Diabetic Foot Ulcer. *J Coll Physicians Surg Pak* 2015; **25**: 721-725 [PMID: 26454386 DOI: 10.2015/JCPSP.721725]

159 **Lullove EJ**, Liden B, Winters C, McEneaney P, Raphael A, Lantis II JC. A multicenter, blinded, randomized controlled clinical trial evaluating the effect of omega-3–rich fish skin in the treatment of chronic, nonresponsive diabetic foot ulcers. Wounds 2021; 33: 169-177 [DOI: 10.25270/wnds/2021.169177]

160 **Del Pino-Sedeño T**, Trujillo-Martín MM, Andia I, Aragón-Sánchez J, Herrera-Ramos E, Iruzubieta Barragán FJ, Serrano-Aguilar P. Platelet-rich plasma for the treatment of diabetic foot ulcers: A meta-analysis. *Wound Repair Regen* 2019; **27**: 170-182 [PMID: 30575212 DOI: 10.1111/wrr.12690]

161 **Golledge J**, Thanigaimani S. Novel therapeutic targets for diabetes-related wounds or ulcers: an update on preclinical and clinical research. *Expert Opin Ther Targets* 2021; **25**: 1061-1075 [PMID: 34873970 DOI: 10.1080/14728222.2021.2014816]

162 **Mahdipour E**, Sahebkar A. The Role of Recombinant Proteins and Growth Factors in the Management of Diabetic Foot Ulcers: A Systematic Review of Randomized Controlled Trials. *J Diabetes Res* 2020; **2020**: 6320514 [PMID: 32733969 DOI: 10.1155/2020/6320514]

163 **Ma C**, Hernandez MA, Kirkpatrick VE, Liang LJ, Nouvong AL, Gordon II. Topical platelet-derived growth factor *vs* placebo therapy of diabetic foot ulcers offloaded with windowed casts: a randomized, controlled trial. *Wounds* 2015; **27**: 83-91 [PMID: 25855851]

164 **Jaiswal SS**, Gambhir RP, Agrawal A, Harish S. Efficacy of topical recombinant human platelet derived growth factor on wound healing in patients with chronic diabetic lower limb ulcers. *Indian J Surg* 2010; **72**: 27-31 [PMID: 23133200 DOI: 10.1007/s12262-010-0005-8]

165 **Liu Y**, Liu Y, Deng J, Li W, Nie X. Fibroblast Growth Factor in Diabetic Foot Ulcer: Progress and Therapeutic Prospects. *Front Endocrinol (Lausanne)* 2021; **12**: 744868 [PMID: 34721299 DOI: 10.3389/fendo.2021.744868]

166 **Richard JL**, Parer-Richard C, Daures JP, Clouet S, Vannereau D, Bringer J, Rodier M, Jacob C, Comte-Bardonnet M. Effect of topical basic fibroblast growth factor on the healing of chronic diabetic neuropathic ulcer of the foot. A pilot, randomized, double-blind, placebo-controlled study. *Diabetes Care* 1995; **18**: 64-69 [PMID: 7698050 DOI: 10.2337/diacare.18.1.64]

167 **Xu J**, Min D, Guo G, Liao X, Fu Z. Experimental study of epidermal growth factor and acidic fibroblast growth factor in the treatment of diabetic foot wounds. *Exp Ther Med* 2018; **15**: 5365-5370 [PMID: 29904416 DOI: 10.3892/etm.2018.6131]

168 **Borys S**, Hohendorff J, Frankfurter C, Kiec-Wilk B, Malecki MT. Negative pressure wound therapy use in diabetic foot syndrome-from mechanisms of action to clinical practice. *Eur J Clin Invest* 2019; **49**: e13067 [PMID: 30600541 DOI: 10.1111/eci.13067]

169 **Liu S**, He CZ, Cai YT, Xing QP, Guo YZ, Chen ZL, Su JL, Yang LP. Evaluation of negative-pressure wound therapy for patients with diabetic foot ulcers: systematic review and meta-analysis. *Ther Clin Risk Manag* 2017; **13**: 533-544 [PMID: 28458556 DOI: 10.2147/TCRM.S131193]

170 **Liu Z**, Dumville JC, Hinchliffe RJ, Cullum N, Game F, Stubbs N, Sweeting M, Peinemann F. Negative pressure wound therapy for treating foot wounds in people with diabetes mellitus. *Cochrane Database Syst Rev* 2018; **10**: CD010318 [PMID: 30328611 DOI: 10.1002/14651858.CD010318.pub3]

171 **Huang J**, Chen J, Xiong S, Huang J, Liu Z. The effect of low-level laser therapy on diabetic foot ulcers: A meta-analysis of randomised controlled trials. Int Wound J 2021; 18: 763-776 [DOI: 10.1111/iwj.13577]

172 **Huang Q**, Yan P, Xiong H, Shuai T, Liu J, Zhu L, Lu J, Shi X, Yang K. Extracorporeal Shock Wave Therapy for Treating Foot Ulcers in Adults With Type 1 and Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Can J Diabetes* 2020; **44**: 196-204.e3 [PMID: 31515158 DOI: 10.1016/j.jcjd.2019.05.006]

173 **Yu Q**, Qiao GH, Wang M, Yu L, Sun Y, Shi H, Ma TL. Stem Cell-Based Therapy for Diabetic Foot Ulcers. *Front Cell Dev Biol* 2022; **10**: 812262 [PMID: 35178389 DOI: 10.3389/fcell.2022.812262]

174 **Ahmed R**, Augustine R, Chaudhry M, Akhtar UA, Zahid AA, Tariq M, Falahati M, Ahmad IS, Hasan A. Nitric oxide-releasing biomaterials for promoting wound healing in impaired diabetic wounds: State of the art and recent trends. *Biomed Pharmacother* 2022; **149**: 112707 [PMID: 35303565 DOI: 10.1016/j.biopha.2022.112707]

175 **Kong M**, Xie K, Lv M, Li J, Yao J, Yan K, Wu X, Xu Y, Ye D. Anti-inflammatory phytochemicals for the treatment of diabetes and its complications: Lessons learned and future promise. *Biomed Pharmacother* 2021; **133**: 110975 [PMID:

33212375 DOI: 10.1016/j.biopha.2020.110975]

176 **Carvalho MTB**, Araújo-Filho HG, Barreto AS, Quintans-Júnior LJ, Quintans JSS, Barreto RSS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. *Phytomedicine* 2021; **90**: 153636 [PMID: 34333340 DOI: 10.1016/j.phymed.2021.153636]

177 **Oguntibeju OO**. Medicinal plants and their effects on diabetic wound healing. *Vet World* 2019; **12**: 653-663 [PMID: 31327900 DOI: 10.14202/vetworld.2019.653-663]

178 **Gallelli G**, Cione E, Serra R, Leo A, Citraro R, Matricardi P, Di Meo C, Bisceglia F, Caroleo MC, Basile S, Gallelli L. Nano-hydrogel embedded with quercetin and oleic acid as a new formulation in the treatment of diabetic foot ulcer: A pilot study. *Int Wound J* 2020; **17**: 485-490 [PMID: 31876118 DOI: 10.1111/iwj.13299]

179 **Majumdar K**, Dtta NK, Dastidar SG, Motohashi N, Shirataki Y. Phytochemical isoflavones against diabetic foot bacteria. *Adv Tradit Med* 2004; **4**: 261-266 [DOI: 10.3742/OPEM.2004.4.4.261]

180 **Thanganadar Appapalam S**, Paul B, Arockiasamy S, Panchamoorthy R. Phytofabricated silver nanoparticles: Discovery of antibacterial targets against diabetic foot ulcer derived resistant bacterial isolates. *Mater Sci Eng C Mater Biol Appl* 2020; **117**: 111256 [PMID: 32919626 DOI: 10.1016/j.msec.2020.111256]

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MINIREVIEWS

Potential role of *Limosilactobacillus fermentum* as a probiotic with anti-diabetic properties: A review

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Abstract

Oxidative stress, inflammation, and gut microbiota impairments have been implicated in the development and maintenance of diabetes mellitus. Strategies capable of recovering the community of commensal gut microbiota and controlling diabetes mellitus have increased in recent years. Some lactobacilli strains have an antioxidant and anti-inflammatory system capable of protecting against oxidative stress, inflammation, and diabetes mellitus. Experimental studies and some clinical trials have demonstrated that *Limosilactobacillus fermentum* strains can beneficially modulate the host antioxidant and anti-inflammatory system, resulting in the amelioration of glucose homeostasis in diabetic conditions. This review presents and discusses the currently available studies on the identification of *Limosilactobacillus fermentum* strains with anti-diabetic properties, their sources, range of dosage, and the intervention time in experiments with animals and clinical trials. This review strives to serve as a relevant and well-cataloged reference of *Limosilactobacillus fermentum* strains capable of inducing anti-diabetic effects and promoting health benefits.

Key Words: Diabetes Mellitus; Gut dysbiosis; Oxidative stress; Probiotics; *Limosilactobacillus fermentum*

Core Tip: This review strives to serve as a relevant and well-cataloged reference of L. fermentum strains with aptitudes of inducing anti-diabetic effects and health-promoting benefits to the host envisaging their wide applicability to diabetes control.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic non-communicable disease that affects millions of people and has become one of the leading causes of death worldwide[1,2]. The Diabetes Atlas published by the International Diabetes Federation estimated that 537 million adults worldwide had type DM in 2021[3]. Associated to this prevalence, clinical management of DM has elevated the costs of the health system, increasing by 316% in the last 15 years[3,4]. One of the etiological factors of this metabolic disorder includes long-term inappropriate diet such as regular consumption of sugary drinks, red meat, and low consumption of whole grains and fiber. In addition, smoking, physical inactivity, history of gestational diabetes or delivery of newborns > 4 kg weight, medications such as statins, thiazides, and beta-blockers, psychosocial stress, and depression have been described as risk factors for DM[5-7].

Clinical features and laboratory findings of DM include changes in body weight, increased blood glucose, insulin resistance, development of lipid metabolism disorder, polyuria, polydipsia, visual disturbances, ketoacidosis, and hyperosmolar non-ketoacidotic syndrome with risk of coma[8,9]. When uncontrolled, diabetes can induce grave complications, including death[10]. Insulin resistance in sensitive tissues such as liver, muscle, and adipose tissue and β-cell dysfunctions are the main factors involved in initiating and progressing the pathophysiology of type 2 DM[5,11]. Moreover, it has been reported that gut microbiota (GM) impairment plays a crucial role in developing DM[12].

DM patients show an altered intestinal microbiota resulting from an increase in opportunistic bacteria and Gram-negative toxin-producing bacteria that alter metabolism energetic[13]. Furthermore, the accumulation of gut-derived pro-inflammatory molecules, including lipopolysaccharide (LPS), peptidoglycans, and flagellin, appear to accelerate the inflammatory response in patients with DM[14]. Deregulation of the GM, also called dysbiosis, promotes intestinal permeability and energy homeostasis changes, causing metabolic endotoxemia, inflammation, hyperglycemia, and hyperlipidemia[15,16]. Dysbiosis impairs the integrity of the intestinal wall and allows the translocation of toxins from the intestinal lumen into the systemic circulation, promoting inflammation, autoimmunity, and oxidative stress that can lead to β-cell destruction or insulin resistance[17,18].

The findings involving the association between gut dysbiosis and DM reinforce the importance of gut-targeting approaches in the treatment of DM[19-21] Strategies capable of recovering the community of commensal GM and controlling DM have recently increased. Probiotic therapy has begun to be used to improve GM composition and management of DM[22,23]. Given this scenario, the identification of new potentially-probiotic strains with anti-diabetic properties is essential for the development of new probiotic products and testing in well-controlled trials.

Among *Lactobacillus* species, strains of *Limosilactobacillus fermentum* (*L. fermentum*) has been reported to exert probiotic properties due to its ability to improve GM composition, reduce blood cholesterol, modulate the intestinal immune system, stimulate the release of immunoglobulin A, reduce intestinal inflammation, and increase the activity of antioxidant enzymes[24-27]. Although early studies have identified anti-diabetic properties in some *L. fermentum* strains, an in-depth review focusing on *L. fermentum* strains as a potential anti-diabetic has not been found in the available literature to the time of this writing[28,29].

This present literature review focuses on the emerging findings of experimental and clinical studies that have used *L. fermentum* supplementation to prevent or treat complications of DM. To investigate the effectiveness of *L. fermentum* more thoroughly, we focus on the type of strain, source of probiotics, dosage, duration of treatment, and the primary outcomes reported.

PROBIOTIC THERAPY IN THE TREATMENT OF METABOLIC DISORDERS

Probiotics are live microorganisms that confer host health benefits when administrated adequately. Probiotics have significant importance in the industrial economy and are among the most consumed food supplements worldwide[30]. Experimental studies and clinical trials have documented that probiotics can modulate the GM, inducing beneficial effects and increasing overall wellness[28,31,32]. Over the last few years, studies on probiotics have been growing sharply due to their beneficial health effects, which have been used as adjuvant therapy for metabolic disorders[33]. A number of preclinical and clinical studies have investigated the effectiveness of probiotics by evaluating the intestinal microbiota after probiotics use, showing promising results in treating metabolic diseases[31].

Impairment in commensal homeostasis of GM and intestinal functional capacity, called gut dysbiosis, is associated with the development of metabolic diseases such as colitis, obesity, liver, obesity, and DM [34]. Thus, a probiotic may be able to relieve GM dysbiosis, through various mechanisms including improvement in the composition and diversity of the GM, induction of immunomodulation, protection against physiological stress, and pathogen suppression[35]. Probiotics also promote health benefits to the host through other mechanisms of action, such as the production of organic acids, including lactic acid and short-chain fatty acids (SCFA) (mainly acetate, propionate, and butyrate)[36,37]. Another mechanism reported is the capacity of probiotics to protect the integrity of the intestinal wall by stimulating mucin production and upregulating tight-junction claudin, occludin, and zonulin protein expression[37]. Furthermore, probiotics are also responsible for producing small molecules with systemic effects essential for maintaining vital functions, such as cortisol, serotonin, gamma-aminobutyric acid (GABA), tryptophan, histamine derivatives, satiety hormones, and conjugated linoleic acid[37].

Coupled with the mechanisms mentioned above, some experimental and clinical evidence has demonstrated that probiotics have anti-inflammatory and antioxidant properties[27,37,38]. This antioxidant capacity results from signaling pathways that produce antioxidant enzymes and molecules, reducing serum and tissues levels of oxidative stress[38,39]. Concerning their anti-inflammatory properties, probiotics have been reported to reduce inflammatory markers, including LPS, tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, as well as to promote an increase of anti-inflammatory markers, such as IL- 10.

LIMOSILACTOBACILLUS FERMENTUM, LEAKY GUT AND DIABETES MELLITUS

Probiotics have shown satisfactory results as an adjunct treatment in DM[40,42]. Both single strain, combined with other foods, and multiple strain probiotics can be used as supplements. Among more effective probiotic strains, the therapeutic potential of *L. fermentum* has been investigated for adjuvant management of DM[40,48,55].

L. fermentum is a Gram-positive, rod- or coccoid-shaped, heterofermentative, and anaerobic or aerotolerant bacteria found in fermented cereals and other fermenting plant materials, dairy products, manure sewage and feces, and the human vagina[41]. The *Lactobacillus* genus is widely used as an intestinal modulator due to its safety and probiotic activity[40]. Among these bacterial groups, *L. fermentum* is a well-studied species, mainly due to its action in improving metabolic function and oxidative stress, which may be considered for DM management[26,27,42].

DM is one of the main metabolic diseases related to leaky gut, oxidative stress, and chronic inflammation. GM impairment has been described in the pathogenesis of DM and metabolic syndrome. Due to the high mortality rate of patients with of DM and this direct relationship with intestinal health, the number of studies involving probiotic therapy has increased in recent years. *L. fermentum* has been proven to alleviate metabolic disorder-related symptoms, including improvement in glucose and insulin levels, control of the lipid profile, to decrease in pro-inflammatory cytokines and to increase antioxidant capacity[27,41,43]. However, these protective responses need to be further investigated in clinical studies to elucidate the responsiveness of *L. fermentum* therapy in DM patients.

Most diabetes treatments, particularly drug therapies, use agents that act directly on signaling pathways to regulate glucose. Because of this, it is pertinent to explore therapies that adjunctively attenuate deregulation of GM, such as probiotics[44]. Among the main harmful effects in GM induced by DM, gram-negative bacteria in the colon increase the concentration of LPS in the lumen. LPS causes high production of free radicals, increasing intestinal permeability and generating a systemic chronic inflammatory process. This pro-inflammatory state is a critical mechanism in the genesis of chronic diseases, such as DM[38,40]. Additionally, GM imbalances observed in DM patients are characterized by changes in the composition of SCFAs, including increasing acetate levels and decreasing butyrate production. As a consequence, there may be acetate excess and reduction of butyrate, caused by dysbiosis, and impaired blood glucose homeostasis[45].

On the other hand, *L. fermentum* manipulation could attenuate GM imbalance, which may decrease DM complications. Considering the inversely proportional relationship between butyrate and acetate levels and the effects of excess acetate on the worsening of DM, keeping these fatty acids in balance

becomes an important way to assist glycemic control[45]. Increased butyrate production by *L. fermentum* regulates acetate production, preventing increased hepatic gluconeogenesis and insulin resistance. Additionally, the increase in butyrate production resulting from *L. fermentum* supplementation may repair enterocyte tight junctions and improve intestinal permeability[46]. Experimental evidence has revealed that increasing levels of SCFA, especially acetate and succinate, decreases cellular damage of enterocytes, leading to a reduction in inflammation state, oxidative stress, and leaky gut in DM-induced rodents[28].

Another antidiabetic property of *L. fermentum* is to maintain normal levels of the intestinal hormone GLP-1[47]. GLP-1 has been shown to stimulate proliferation and prevent apoptosis of pancreatic beta cells, upregulating insulin synthesis and promoting a reasonable glycemic control[29,36]. In the liver, GLP1 decreases gluconeogenesis and stimulates glycolysis, contributing to reducing glycemic levels in individuals with DM. The main consequence of reducing these peptides is the exacerbation of hunger, the search for palatable food, and the preference for hypercaloric foods, which can be a predisposing factor for developing obesity and insulin resistance[30]. Leaky gut also generates chronic low-grade inflammation in organs such as the liver, skeletal muscle, and adipose tissue, causing metabolic changes such as hyperglycemia and dyslipidemias. *L. fermentum* also promotes benefits in these organs because it stimulates the synthesis of the fasting-induced adipose factor, a protein that regulates the function of the LPL enzyme and prevents hepatic steatosis and dyslipidemia, common in diabetic subjects[48]. Therefore, it is suggested that *L. fermentum* may improve intestinal permeability, normalize GLP-1 Levels, and reduce DM complications.

Another important action of *L. fermentum* is to reduce oxidative stress and glycation. Studies indicate that pathophysiological findings of DM, including macular degeneration, vascular endothelial injury, hepatic fibrosis, renal failure, are related to the glycation process. This process occurs when circulating glucose binds to proteins, inactivating them and increasing inflammatory cytokines such as interferon-gamma (IFN-γ), IL-6, and IL-4. The main biochemical marker for glycation is glycated hemoglobin (HB1ac), but this process can occur with any protein, including antioxidant enzymes. When glycation events occur more expressively, oxidative stress is even higher due to the increase in reactive oxygen species (ROS) and inactivation of the enzymatic antioxidant systems, such as superoxide dismutase (SOD) and glutathione peroxidase[40,49].

Conversely, the administration of *L. fermentum* decreased the glycation events and oxidative stress through the increasing production of ferulic acid (FA). This potent antioxidant metabolite can significantly reduce ROS formation and prevent glycation events. This mechanism is related to decreasing inflammatory markers, Hb1ac, and serum glucose. High levels of FA are also related to lower cardiometabolic risk in diabetic individuals[40,44].

To evaluate the effectiveness of *L. fermentum,* the following sections refer to the findings on the antidiabetic properties of different strains of *L. fermentum,* investigated in preclinical and clinical studies.

ANTI-DIABETIC PROPERTIES OF DIFFERENT STRAINS OF *LIMOSILACTOBACILLUS FERMENTUM*

We investigated studies that analyzed the role of *L. fermentum* administered singly or combined with other therapies to alleviate DM complications. Among ten of the studies included, nine evaluated anti-diabetic properties in experimental studies using rats or mice. Only one clinical study assessed the anti-diabetic potential of probiotic intervention in women with gestational DM. Since the majority of beneficial effects following administration of *L. fermentum* come from animal studies, this present review investigated emerging findings of their potential role in DM management. The characteristics of the studies and the primary outcomes are summarized in Table 1 and Table 2, respectively.

L. fermentum LLB3

An experimental study revealed that treatment with *L. fermentum* LLB3 isolated from the bamboo shoot pickle and offered in fermented bitter melon (*Momordica charantia*), in a concentration of 1×10^7 CFU during 4 wk, reduced fasting glucose and postprandial blood glucose levels and increased SOD enzyme activity in rats subjected to type 2 DM induced by streptozotocin (STZ)[50]. This suggests that *L. fermentum* LLB3 might be considered an adjuvant therapy to attenuate type 2 DM-related symptoms[50].

L. fermentum HP3

Administration of a fermented *Hericium erinaceus* juice containing 10^9 CFU/mL of *L. fermentum* HP3 for 12 wk reduced weight gain, increased insulin level, and reduced hyperglycemia in diabetic mice induced by STZ[51]. In addition, treated mice showed lower levels of inflammatory cytokines, including IL-6, IL-17, and IFN-γ[51], suggesting that fermented *Hericium erinaceus* juice can be used as nutritional manipulation in the treatment of type 2 DM.

Table 1 Characteristics of the studies testing the anti-diabetic effect of *Limosilactobacillus fermentum* strains

Ref.	Type of study	Experimental groups	Source of probiotics	Dosage of probiotic	Duration of treatment
Hartajanie *et al*[50], 2020	Experimental: 24 male Sprague-Dawley rats at 8 wk and weighing 170-200 g	Diabetic group; Diabetic group + acarbose; Diabetic group + bitter melon; Diabetic group + fermented bitter melon	*L. fermentum* LLB3 was isolated from the bamboo shoot pickle	1×10^7 CFU *L. fermentum* LLB3	4 wk
Hu *et al* [35], 2019	Experimental: 4-wk-old male Kunming mice (18 ± 2 g) were used	Normal control group; Diabetic group; Positive drug control group; Diabetic group + fructose 1 6-bisphosphatase (low dose); Diabetic group + 1-Deoxynojirimycin (middle dose); Diabetic group + 1-Deoxyn-ojirimycin (high dose)	All probiotics were purchased from the Guangdong culture collection center	5×10^4 CFU/mL of each activated strain (*L. plantarum* + *L. fermentum*, *L. plantarum* + *L. mesenteroides*, *L. plantarum* + *S. cerevisiae*, *L. fermentum* + *L. mesenteroides*, *L. fermentum* + *S. cerevisiae*, and *L. mesenteroides* + *S. cerevisiae*)	4 wk
Chaiyasut *et al*[51], 2018	Experimental: male *Wistar* rats	Control group; Control group + *L. fermentum*; Control group + fermented *H. erinaceus* juice; Diabetic group; Diabetic group pretreatment and posttreatment treated with fermented *H. erinaceus* juice, *L. fermentum*, and insulin	*L. fermentum* HP3 was isolated from fermented Thai foods	*L. fermentum* HP3 in a concentration of 10^9 CFU/mL. *L. fermentum* HP3 was used with *H. Erinaceus* Juice	12 wk
Guilbaud *et al*[52], 2020	Experimental: 30 mice with 6 wk of age	Wild-type group;Wild-type group + *L. fermentum*;Diabetic group;Diabetic group + *L. fermentum*	Isolated from a fecal sample of one-year-old healthyE-stonian child	*L. fermentum* ME-3 in a concentration of 10^{10} CFU per 400 μL H_2O	12 wk
Archer *et al* [48], 2021	Experimental: 40 female *Wistar* rats	Control group; Diabetic group + high-fat diet; Diabetic group + high-fat diet + *L. fermentum*. MCC2759; Diabetic group + high-fat diet + *L. fermentum*. MCC2760	Isolated from fecal (*L. fermentum*. MCC2759) and from curd (*L. fermentum*. MCC2760)	Both isolated probiotics were offered in a concentration of 1×10^9 CFU/mL	4 wk
Ai *et al*[31], 2021	Experimental: 160 Male C57BL/6J mice with 6 wk of age	Control group; Diabetic group + high-fat diet; Diabetic group + defatted rice bran unfermented extracts; Diabetic group + pioglitazone; Diabetic group + high-dose of defatted rice bran fermentation extracts; Diabetic group + low-dose of defatted rice bran fermentation extracts	Isolated from Chinese rice noodle wastewater	The study evaluated the role of *L. fermentum* MF423. Dose of 100 μg/mL of defatted rice bran unfermented extracts	8 wk
Yadav *et al* [54], 2018	Experimental: 70 male *Wistar* rats with 8 ws old	Normal control group; Diabetic control group; Diabetic + normal diet supplemented with milk; Diabetic + *L. rhamnosus* MTCC5957; Diabetic + *L. rhamnosus* MTCC5897; Diabetic + *L. fermentum* MTCC 5898; Diabetic + *L.rhamnosus* 5957 and 5958 and *L. fermentum* MTCC 5898	The probiotics *L. rhamnosus* MTCC: 5957 and *L. rhamnosus* MTCC: 5897 were isolated from household curds. The probiotic *L. fermentum* MTCC: 5898 was isolated from the feces of breastfed human infants	All probiotic strains were offered in a dosage of 1×10^9 CFU	6 wk
Yousaf *et al* [55], 2016	Experimental: female mice of 6-8 wk, with an initial body weight of 21-23 g	Normal healthy mice; Diabetic mice; Diabetic mice + *Momordica charanti*; Diabetic mice + *Eugenia Jambolana*; Diabetic mice + *L. Fermentum*; Diabetic mice + *L. Fermentum* + Momordicacharanti + *Eugenia Jambolana*; Diabetic mice + Glucophage	*L. fermentum* fruit extracts of *Eugenia Jambolana* and *Momordica charantia* were isolated from local yogurt samples (Lahore, Pakistan)	*Momordica charantia* 200 mg/kg, and *Eugenia Jambolana* 100 mg/kg. The authors did not inform the concentration of *L. fermentum* (Gene Bank Accession KJ754019)	3 wk
Balakumar *et at*[49], 2018	Experimental: adult male C57BL/6J mice (age 8-10 wk)	Normal pellet diet; High-fat diet; High-fat diet + *L. rhamnosus*; High-fat diet + *L. plantarum* MTCC5690; High-fat diet + *L. fermentum* MTCC5689; High-fat diet + metformim; High-fat diet + vildagliptin	Isolated from Indian gut (Karnal, India)	*Lactobacillus* MTCC 5690 and MTCC 5689 in a concentration of 1.5×10^9 colonies/mouse/d	24 wk
Babadi *et al* [30], 2018	Clinical: primigravid women aged between 18 and 40 years, between the 24th and 28th week of gestation, diagnosed with gestational diabetes	Placebo group; Probiotic group	Probiotic supplements were produced by LactoCare®, Zisttakhmir Company (Tehran, Iran)	Probiotic capsule containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *L. fermentum* in a dosage of 2×10^9 CFU/g	6 wk

	mellitus

L. fermentum: *Lactobacillus fermentum*; *L. rhamnosus*: *Lactobacillus rhamnosus*; *L. plantarum*: *Lactobacillus plantarum*; *S. cerevisiae*: *Saccharomyce cerevisae*; *L. mesenteroides*: *Leuconostoc mesenteroides*; *H. erinaceus*: *Hericium erinaceus*.

Table 2 Primary outcomes of the studies testing the anti-diabetic effect of *Limosilactobacillus fermentum* strains

Ref.	Primary end-points
Hartajanie *et al*[50], 2020	↓ The fasting blood glucose; ↓ Postprandial blood glucose; ↑ In SOD concentrations
Hu *et al*[35], 2019	↓ Blood glucose levels; ↓ Insulin levels; Reversed insulin resistance; Improved serum lipid levels; Relieved gut dysbiosis
Chaiyasut *et al*[51], 2018	↓ Weight Gain; Improved insulin levels (↑ insulin); Recovery progress of hyperglycemia; ↓ HbA1c level (only with cointerventions); ↓ Inflammatory cytokines level
Guilbaud *et al*[52], 2020	↓ Weight Gain; ↓ Glycemic response 60-120 min; ↑ In HbA1c; ↓Weight of liver; ↓ FL-furosine levels in kidney ↓The expression of *TNF-α;* ↓The TG concentrations in liver; ↓ HDL and Non-HDL; Lower lipid droplets in liver.
Archer *et al*[48], 2021	↓ Blood glucose levels; Improved insulin levels (↑ insulin); ↓ levels of cholesterol, triglycerides, and LDL-C; ↓ The expression levels of *TNF-α*, and ↑ expression of *IL-10*; ↓ Expression of the *TLR4* receptor, ↑ Expression of tight junction protein *ZO-1*, endocannabinoid receptor *CB2* and GLP1, and ↑ Expression of *GLUT4* in MAT and muscle tissue; Showed accumulation of neutrophils around the portal tracts in liver tissue, and reduction in the glomerular injury in kidney sections
Ai *et al*[31], 2021	Inhibit the degree of weight loss; ↓ The fasting blood glucose; ↓ Blood glucose levels; ↓ Levels of total cholesterol and LDL and ↑ HDL levels; Ameliorate the damage to liver cells and significantly reduced the accumulation of lipid droplets; Upregulated the levels of SOD, T-AOC and GSH-PX, and reversed elevation of MDA; ↓ Damage in composition of gut microbiota[1]
Yadav *et al*[54], 2018	Inhibit the degree of weight loss; ↓ The fasting blood glucose; ↓ Consumption of food and liquids; ↑ In oral glucose tolerance; ↑ In liver weight; Improved insulin levels (↑ Insulin); ↓ HbA1c level; ↑ CAT, SOD activity in kidney and liver; ↓ Serum levels of total cholesterol, LDL-C, VLDL-C and triglycerides; ↓ The serum inflammatory index, cytokine levels (IL-6 and TNF-α); ↓ In the expression of the genes *G6Pase* and *pepck* in the liver
Yousaf *et al*[55], 2016	↑ Body weight; ↓ Blood glucose levels; Lipid profile: no effect on cholesterol, ↓ tryglyceride, LDL, slight increase in the level of HDL
Balakumar *et al*[49], 2018	↓ Body weight; ↓ Blood glucose levels; ↑ In oral glucose tolerance; ↓ HbA1c level; Improved insulin levels (↓ Insulin); ↑ levels of GLP-1; ↓ Cholesterol, triglyceride and LDL levels; ↑ HDL level; ↓ Plasma DX-4000–FITC; ↑ mRNA expression of epithelial tight junction *occludin* and *ZO-1*; ↓ Serum levels of LPS; ↓ Proinflammatory gene expression profiles *(IL6* and *TNFα*), ↑ *adiponectin* gene expression; ↓ Gene expression profiles of endoplasmic reticulum stress
Babadi *et al*[30], 2018	Downregulated gene expression of *TNF-α*; ↓ The fasting blood glucose; ↓ Serum insulin level; ↓ Insulin resistance; ↑ Insulin sensitivity; ↓ Levels of triglycerides, VLDL-cholesterol and total / HDL-cholesterol ratio, and ↑ levels of HDL-cholesterol; ↓ In plasma MDA; ↑ In plasma NO and total antioxidant capacity

[1]These results were obtained by rice bran fermented with Lactobacillus fermentum MF423.

SOD: Superoxide dismutase; HbA1c: Glycayed hemoglobin A; TNF-α: Tumor necrosis factor-alpha; TG: Triglyceride; HDL- C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; VLDL-C: Very-low-density lipoprotein cholesterol; IL-6: Interleukin-6; IL-10: Interleukin-10; TLR4: Toll-like receptor 4; ZO-1: Zonula occludens-1; CB2: Cannabinoid receptor type 2; GLP1: Glucagon-like peptide-1; GLUT4: Glucose transporter type 4; MAT: Mesenteric adipose tissue; T-AOC: Total antioxidant capacity; GSH-PX: Glutathione peroxidase; MDA: Malondialdehyde; CAT: Catalase; G6Pase: Glucose 6-phosphatase; Pepck: Phosphoenolpyruvate carboxykinase; FITC: Fluorescein isothiocyanate-dextran; LPS: Lipopolysaccharide; NO: Nitric oxide.

L. fermentum ME-3

A previous preclinical study investigated the anti-diabetic effect of *L. fermentum* ME-3 in genetically diabetic mice[52]. *L. fermentum* ME-3 was administered in mice at 6 wk of age, in a concentration of 10^{10} CFU, for 12 wk[52]. The treatment with *L. fermentum* ME-3 reduced body weight, inhibited expression of TNF-α, but did not improve glycemic control[52]. In addition, supplementation with *L. fermentum* ME-3 reduced the formation of glycation products, including FL-furosine levels in the kidney. However, the researchers found an increase in HbA1c, another marker of early glycation. The authors noted that while HbA1c reflects early glycation mainly in red blood cells, FL-furosine provides information on the extent of early glycation in fluids, tissues, and organs and offers a broader view of the early glycation status of the whole organism[52]. In summary, *L. fermentum* ME-3 has the therapeutic potential to reduce the formation of some glycation products in kidneys and attenuate some typical type 2 DM-related symptoms.

L. fermentum MCC2759 and MCC2760

Recently, an Indian research group analyzed the activity of the probiotic *L. fermentum* MCC2759 and MCC2760 on intestinal markers of inflammation using a high-fat diet model associated with the STZ-

induced diabetic model[48]. Both *L. fermentum* strains were administered in a concentration of 1 × 10^9 CFU/mL for 4 wk. The main findings of the study revealed that diabetic female rats treated with *L. fermentum* MCC2759 and MCC2760 reduced blood glucose levels, increased insulin levels, and improved the lipid profile[48]. Coupled with biochemical changes, *L. fermentum* administration downregulated TNF-α mRNA and up-regulated mRNA IL-10 in the intestine, liver, mesenteric adipose tissue, and muscle, suggesting that the anti-diabetic effect promoted by *L. fermentum* MCC2759 and MCC2760 can be associated with a decrease in inflammatory markers[48]. In addition, *L. fermentum* MCC2759 and MCC2760 administration modulated other gene expressions, such as reduced expression of Toll-like receptor 4, enhanced expression of tight junction protein ZO-1, endocannabinoid receptor CB2 and GLP1, glucose transporter type 4 in mesenteric adipose tissue and muscle tissue. The results demonstrated that *L. fermentum* MCC2759 and MCC2760 might be a potential probiotic in treating type 2 DM.

L. fermentum MF423

L. fermentum MF423 is a strain isolated from Chinese rice noodle wastewater[31]. The authors analyzed adverse effects triggered by an experimental model of type 2 DM induced by STZ and tested the effectiveness of different therapies, including supplementation with unfermented extracts of defatted rice bran, high and low doses of defatted rice bran fermented by *L. fermentum* MF423, and drug intervention (pioglitazone)[31]. Mice receiving a high dose (1 g/kg) of defatted rice bran fermentation extracts containing *L. fermentum* MF423 for 8 wk evidenced weight loss and reduced fasting blood glucose, lipid accumulation, and liver cells damage[31]. Moreover, probiotic groups intensified antioxidant activity in diabetic mice through up-regulation levels of SOD, total antioxidant capacity (T-AOC), and reversed elevation of malondialdehyde (MDA) in the liver[31]. It is important to mention that no effects were found in animals treated only by unfermented extracts of rice bran, highlighting the antioxidant activity of *L. fermentum* MF423.

To complete the evaluation of the therapeutic potential of *L. fermentum* MF423, the authors investigated the role of this probiotic in the modulation of GM. Diabetic rats treated either to high dose of defatted rice fermented by *L. fermentum* or pioglizatone showed GM composition similar to the control group, compared to untreated diabetic animals[31]. The relative abundances of *Bacteroidetes* (20%) and *Firmicutes* (40%) were increased in both mentioned groups compared to a diabetic group without treatment[31]. A decreased abundance of *Firmicutes* can be found in diabetic patients compared to their non-diabetic counterparts[53]. These two major phyla may play an essential role in hyperglycemia, hyperlipidemia, and inflammation. Moreover, probiotic treatment increased the relative abundance of SCFA-producing bacteria in diabetic mice, including *Lactobacillus, Parabacteroides, norank_f_Ruminococcaceae, Ruminococcus_torques*_group, and *Alloprevotella*. Interestingly, a decrease in the genus *Lactobacillus* was significant in diabetic mice, while treatment with defatted rice bran fermented by *L. fermentum* MF423 increased its abundance, similar to control mice[31]. *L. fermentum* is known for its probiotic role in food consumption, which could modify abnormalities in intestinal microbes and retard hyperglycemia. In conclusion, defatted rice bran fermentation by *L. fermentum* MF423 Lessened damage to the structure and function of GM induced by type 2 DM.

L. fermentum MTCC: 5898

Probiotic fermented milk prepared using different probiotic strains, including *L. rhamnosus* MTCC: 5957, *L. rhamnosus* MTCC: 5897, and *L. fermentum* MTCC: 5898, were evaluated in an experimental study[54]. Probiotic strains were offered independently or in combination for treating STZ induced type 1 DM in male *Wistar* rats. All probiotic strains were provided in a dosage of 1 × 10^9 CFU for 6 wk. The study demonstrated that the diabetic rats who received fermented milk containing *L. fermentum* MTCC: 5898 had less weight loss, improved glucose metabolism by reducing fasting blood glucose, HbA1c associated with increased insulin level, reduced diabetic dyslipidemia, and attenuated inflammation status through reduction of IL-6 and TNF-α[54]. In addition, supplementation with *L. fermentum* MTCC: 5898 showed antioxidant properties by increasing catalase (CAT) and SOD activities in the kidney and liver[54]. Moreover, administration of probiotics reduced mRNA expression of phosphoenolpyruvate carboxykinase and Glucose 6-phosphatase genes that code the key enzymes of the gluconeogenesis pathway[54]. Compared to other lactobacilli strains, rats receiving *L. fermentum* MTCC: 5898 displayed the most effective responses including oral glucose tolerance, serum insulin, serum, liver CAT, serum triglycerides, VLDL[54]. Therefore, it is suggested that daily consumption of probiotic fermented milk, especially *L. fermentum* MTCC: 5898, may be effective in attenuating complications of type 1 DM.

L. fermentum MTCC 5690 and MTCC 5689

L. fermentum MTCC 5690 and MTCC 5689 were isolated from the Indian gut and used to treat high-fat diet-induced type 2 DM mice[49]. The present study compared the anti-diabetic effect of *L. fermentum* MTCC 5690 and MTCC 5689 to other probiotics (*Lactobacillus rhamnosus*, *Lactobacillus plantarum* MTCC5690) and drug intervention (metformin, vildagliptin). *L. fermentum* MTCC 5690 and MTCC 5689 were administered in a concentration of 1.5 × 10^9 colonies/mouse/day for 24 wk[49]. Both probiotics and drugs groups reduced body weight, improved oral glucose tolerance, and reduced fasting glucose

and Hb1Ac levels in diabetic mice[49]. Concerning insulin levels, probiotic groups contributed to normalizing levels of this hormone, which approximated the levels observed in the control group[49]. In addition, a significant reduction of insulin levels was found in the vildagliptin group compared with other groups, which may be considered a possible adverse mild effect of this drug. Furthermore, all treatment groups improved lipid profile by reduction of levels of cholesterol, triglycerides, LDL, associated with an increase in HDL levels.

Additionally, the study evaluated the gut integrity after 24 wk of treatment. Probiotic treatment and drug therapy were able to reduce damage in gut integrity, which may contribute to normalizing gut permeability[49]. The authors quantified mRNA expression of epithelial tight junction occludin and ZO-1 and LPS levels. All the probiotic and anti-diabetic drugs increased gene expression of the intestinal tight junction occludin and ZO-1[49]. To evaluate endotoxemia state and intestinal barrier integrity, probiotic treatments decreased LPS levels and pro-inflammatory cytokines IL-6 and TNF-α in mice subjected to type 2 DM[49]. In addition, the authors verified the effectiveness of treatments in attenuating endoplasmic reticulum stress of skeletal muscle of diabetic mice. Results showed that both probiotic and drug therapies reduced endoplasmic reticulum stress markers[49]. The findings suggested that *L. fermentum* MTCC 5690 and MTCC 5689 act like anti-diabetic drugs, highlighting the therapeutic potential of these probiotic strains in alleviating type 2 DM complications.

Non mentioned strain of L. fermentum

The role of *L. fermentum* fruit extracts of *Syzygium cumini* and *Momordica charantia*, isolated from yogurt samples (Pakistan) on STZ induced DM mice, was previously investigated[55]. *L. fermentum* and the extracts were administered individually as well as in combination with DM-induced mice for 3 wk. Results were compared with mice that received drug intervention (Glucophage). Administration of probiotics and natural extracts improved body weight, and reduced blood glucose levels and both results were similar with the Glucophage group[55]. Concerning lipid profile, *L. fermentum* and natural extracts improved almost all lipid profile parameters, including reduced triglycerides, LDL, and increased HDL serum levels[55]. The study demonstrated that Glucophage treatment might affect some parameters, such as increased total cholesterol, triglycerides, and LDL concentration. These findings showed that *L. fermentum* and natural extracts have hypoglycemic and hypolipidemic activity, which may reduce DM complications.

Another experimental study demonstrated that mixed probiotics containing *L. fermentum* could reverse insulin resistance, reduce blood glucose levels, and improve lipid profile in STZ induced DM in old male Kunming mice after 4 wk of treatment[35]. In addition, the authors showed a significant impact of the supplementation of *L. fermentum* on the relief of gut dysbiosis, lowering the damage in the composition of GM[35]. However, the authors did not specify which strain of *L. fermentum* was administered, limiting the understanding of these effects.

Regarding clinical data, we found only one randomized, double-blind, placebo-controlled trial that evaluated the effectiveness of *L. fermentum* in attenuating complications of DM[30]. This study was carried out to assess the effects of probiotic supplementation on genetic and metabolic profiles in patients with gestational DM, aged 18-40 years (at weeks 24-28 of gestation)[30]. Participants were randomly divided into two groups: a control group and a probiotic group, made up of women who received a probiotic capsule containing *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, L. fermentum* (2×10^9 CFU/g each) for 6 wk[30]. However, the authors did not inform the specific strain of *L. fermentum* used, limiting the interpretation of results. The probiotic group showed lower levels of fasting blood glucose serum insulin, reduced insulin resistance, and significantly increased insulin sensitivity compared with the control group[30]. In addition, probiotic supplementation decreased triglycerides, VLDL, and increased HDL levels compared with the control group. Additionally, probiotic administration reduced plasma MDA, and an elevation in plasma nitric oxide and T-AOC was found compared with the control group. Therefore, the probiotic treatment showed great therapeutic potential in alleviating complications found in women with gestational DM. Future clinical studies are needed to investigate further the specific strains of *L. fermentum* to elucidate which strains are more effective in attenuated DM.

CONCLUSION

This literature review showed that *L. fermentum* is a promising strain for the management of DM (Figure 1). Evidence from experimental and clinical study verified that *L. fermentum* supplementation contributed to normalizing body weight, reduced blood glucose and fasting blood glucose levels, reduced insulin resistance, and improved lipid profile. Coupled with these biochemical changes, *L. fermentum* therapy showed anti-oxidant and anti-inflammatory properties, which contributed to alleviating related symptoms of DM. However, the heterogeneities of studies, including variations in dosage, and duration of treatment, limit the elucidation of the most effective way to use *L. fermentum* as adjuvant therapy of DM. Moreover, it is relevant to explore the effectiveness of co-intervention with *L. fermentum* associated with bioactive compounds with antioxidant and anti-inflammatory properties,

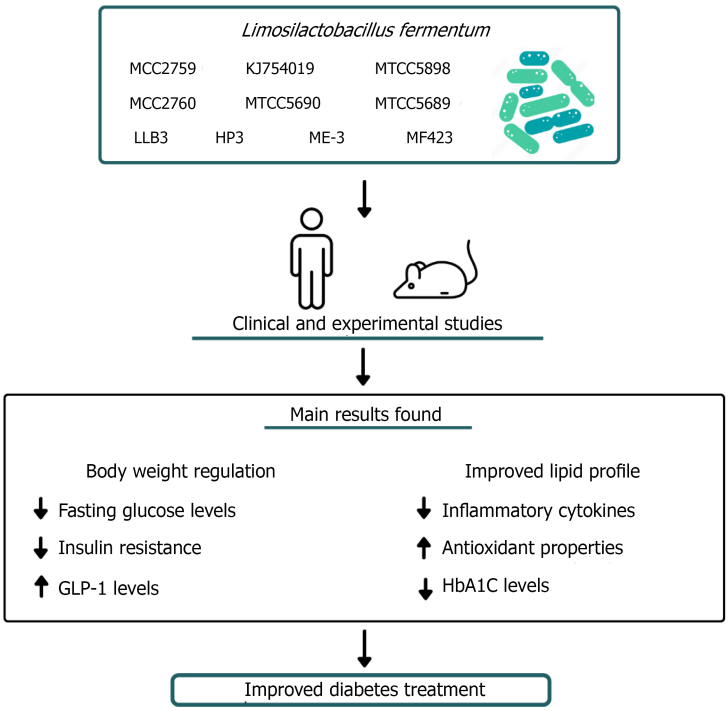

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Figure 1 Schematic drawing showing that *Limosilactobacillus fermentum* exert an anti-diabetic effect.

such as quercetin and resveratrol [15]. We also highlight that most of the available data came from preclinical studies, hence, therapeutic potential of different strains of *L. fermentum* in minimizing complications of DM needs to be further investigated in randomized, double-blind, placebo-controlled trials to confirm these findings in human studies.

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FOOTNOTES

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REFERENCES

1 **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]

2 **Tinajero MG**, Malik VS. An Update on the Epidemiology of Type 2 Diabetes: A Global Perspective. *Endocrinol Metab Clin North Am* 2021; **50**: 337-355 [PMID: 34399949 DOI: 10.1016/j.ecl.2021.05.013]

3 **Sun H**, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022; **183**: 109119 [PMID: 34879977 DOI: 10.1016/j.diabres.2021.109119]

4 **Jin Q**, Ma RCW. Metabolomics in Diabetes and Diabetic Complications: Insights from Epidemiological Studies. *Cells* 2021; **10** [PMID: 34831057 DOI: 10.3390/cells10112832]

5 **Zheng Y**, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* 2018; **14**: 88-98 [PMID: 29219149 DOI: 10.1038/nrendo.2017.151]

6 **Taylor R**. Type 2 diabetes: etiology and reversibility. *Diabetes Care* 2013; **36**: 1047-1055 [PMID: 23520370 DOI: 10.2337/dc12-1805]

7 **Lee E**. Diabetes: Screening, Diagnosis, and Prevention of Type 2 Diabetes. *FP Essent* 2021; **504**: 16-21 [PMID: 33970587]

8 **Roden M**. [Diabetes mellitus: Definition, classification and diagnosis]. *Wien Klin Wochenschr* 2012; **124** Suppl 2: 1-3 [PMID: 23250457 DOI: 10.1007/s00508-012-0269-z]

9 **Cloete L**. Diabetes mellitus: an overview of the types, symptoms, complications and management. *Nurs Stand* 2022; **37**: 61-66 [PMID: 34708622 DOI: 10.7748/ns.2021.e11709]

10 **Glovaci D**, Fan W, Wong ND. Epidemiology of Diabetes Mellitus and Cardiovascular Disease. *Curr Cardiol Rep* 2019; **21**: 21 [PMID: 30828746 DOI: 10.1007/s11886-019-1107-y]

11 **Ceriello A**, Prattichizzo F. Variability of risk factors and diabetes complications. *Cardiovasc Diabetol* 2021; **20**: 101 [PMID: 33962641 DOI: 10.1186/s12933-021-01289-4]

12 **Festi D**, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. *World J Gastroenterol* 2014; **20**: 16079-16094 [PMID: 25473159 DOI: 10.3748/wjg.v20.i43.16079]

13 **Chakaroun RM**, Massier L, Kovacs P. Gut Microbiome, Intestinal Permeability, and Tissue Bacteria in Metabolic Disease: Perpetrators or Bystanders? *Nutrients* 2020; **12** [PMID: 32295104 DOI: 10.3390/nu12041082]

14 **Woldeamlak B**, Yirdaw K, Biadgo B. Role of Gut Microbiota in Type 2 Diabetes Mellitus and Its Complications: Novel Insights and Potential Intervention Strategies. *Korean J Gastroenterol* 2019; **74**: 314-320 [PMID: 31870137 DOI: 10.4166/kjg.2019.74.6.314]

15 **Sampaio KB**, do Nascimento YM, Tavares JF, Cavalcanti MT, de Brito Alves JL, Garcia EF, de Souza EL. Development and *in vitro* evaluation of novel nutraceutical formulations composed of Limosilactobacillus fermentum, quercetin and/or resveratrol. *Food Chem* 2021; **342**: 128264 [PMID: 33041168 DOI: 10.1016/j.foodchem.2020.128264]

16 **Slingerland AE**, Schwabkey Z, Wiesnoski DH, Jenq RR. Clinical Evidence for the Microbiome in Inflammatory Diseases. *Front Immunol* 2017; **8**: 400 [PMID: 28446909 DOI: 10.3389/fimmu.2017.00400]

17 **Arora T**, Bäckhed F. The gut microbiota and metabolic disease: current understanding and future perspectives. *J Intern Med* 2016; **280**: 339-349 [PMID: 27071815 DOI: 10.1111/joim.12508]

18 **Sohail MU**, Althani A, Anwar H, Rizzi R, Marei HE. Role of the Gastrointestinal Tract Microbiome in the Pathophysiology of Diabetes Mellitus. *J Diabetes Res* 2017; **2017**: 9631435 [PMID: 29082264 DOI: 10.1155/2017/9631435]

19 **Cabello-Olmo M**, Araña M, Urtasun R, Encio IJ, Barajas M. Role of Postbiotics in Diabetes Mellitus: Current Knowledge and Future Perspectives. *Foods* 2021; **10** [PMID: 34359462 DOI: 10.3390/foods10071590]

20 **Bastos RMC**, Rangel ÉB. Gut microbiota-derived metabolites are novel targets for improving insulin resistance. *World J Diabetes* 2022; **13**: 65-69 [PMID: 35070060 DOI: 10.4239/wjd.v13.i1.65]

21 **Xia F**, Wen LP, Ge BC, Li YX, Li FP, Zhou BJ. Gut microbiota as a target for prevention and treatment of type 2 diabetes: Mechanisms and dietary natural products. *World J Diabetes* 2021; **12**: 1146-1163 [PMID: 34512884 DOI: 10.4239/wjd.v12.i8.1146]

22 **Xi Y**, Xu PF. Diabetes and gut microbiota. *World J Diabetes* 2021; **12**: 1693-1703 [PMID: 34754371 DOI: 10.4239/wjd.v12.i10.1693]

23 **Patterson E**, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, Stanton C. Gut microbiota, obesity and diabetes. *Postgrad Med J***92**: 286-300 [PMID: 26912499 DOI: 10.1136/postgradmedj-2015-133285]

24 **Liang T**, Wu L, Xi Y, Li Y, Xie X, Fan C, Yang L, Yang S, Chen X, Zhang J, Wu Q. Probiotics supplementation improves hyperglycemia, hypercholesterolemia, and hypertension in type 2 diabetes mellitus: An update of meta-analysis. *Crit Rev Food Sci Nutr* 2021; **61**: 1670-1688 [PMID: 32436397 DOI: 10.1080/10408398.2020.1764488]

25 **Rittiphairoj T**, Pongpirul K, Janchot K, Mueller NT, Li T. Probiotics Contribute to Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. *Adv Nutr* 2021; **12**: 722-734 [PMID: 33126241 DOI: 10.1093/advances/nmaa133]

26 **Jang YJ**, Kim WK, Han DH, Lee K, Ko G. *Lactobacillus fermentum* species ameliorate dextran sulfate sodium-induced colitis by regulating the immune response and altering gut microbiota. *Gut Microbes* 2019; **10**: 696-711 [PMID: 30939976 DOI: 10.1080/19490976.2019.1589281]

27 **Yadav R**, Khan SH, Mada SB, Meena S, Kapila R, Kapila S. Consumption of Probiotic Lactobacillus fermentum MTCC: 5898-Fermented Milk Attenuates Dyslipidemia, Oxidative Stress, and Inflammation in Male Rats Fed on Cholesterol-Enriched Diet. *Probiotics Antimicrob Proteins* 2019; **11**: 509-518 [PMID: 29754388 DOI: 10.1007/s12602-018-9429-4]

28 **de Luna Freire MO**, do Nascimento LCP, de Oliveira KÁR, de Oliveira AM, Dos Santos Lima M, Napoleão TH, da Costa Silva JH, Lagranha CJ, de Souza EL, de Brito Alves JL. Limosilactobacillus fermentum Strains with Claimed Probiotic Properties Exert Anti-oxidant and Anti-inflammatory Properties and Prevent Cardiometabolic Disorder in Female Rats Fed a High-Fat Diet. *Probiotics Antimicrob Proteins* 2021 [PMID: 34817804 DOI: 10.1007/s12602-021-09878-1]

29 **Kim JE**, Lee JY, Kang CH. *Limosilactobacillus fermentum* MG4295 Improves Hyperglycemia in High-Fat Diet-Induced Mice. *Foods* 2022; **11** [PMID: 35053962 DOI: 10.3390/foods11020231]

30 **Babadi M**, Khorshidi A, Aghadavood E, Samimi M, Kavossian E, Bahmani F, Mafi A, Shafabakhsh R, Satari M, Asemi Z. The Effects of Probiotic Supplementation on Genetic and Metabolic Profiles in Patients with Gestational Diabetes Mellitus: a Randomized, Double-Blind, Placebo-Controlled Trial. *Probiotics Antimicrob Proteins* 2019; **11**: 1227-1235 [PMID: 30535534 DOI: 10.1007/s12602-018-9490-z]

31 **Ai X**, Wu C, Yin T, Zhur O, Liu C, Yan X, Yi C, Liu D, Xiao L, Li W, Xie B, He H. Antidiabetic Function of *Lactobacillus fermentum* MF423-Fermented Rice Bran and Its Effect on Gut Microbiota Structure in Type 2 Diabetic Mice. *Front Microbiol* 2021; **12**: 682290 [PMID: 34248898 DOI: 10.3389/fmicb.2021.682290]

32 **Clarke TC**, Black LI, Stussman BJ, Barnes PM, Nahin RL. Trends in the use of complementary health approaches among adults: United States, 2002-2012. *Natl Health Stat Report* 2015; 1-16 [PMID: 25671660]

33 **Kim SK**, Guevarra RB, Kim YT, Kwon J, Kim H, Cho JH, Kim HB, Lee JH. Role of Probiotics in Human Gut Microbiome-Associated Diseases. *J Microbiol Biotechnol* 2019; **29**: 1335-1340 [PMID: 31434172 DOI: 10.4014/jmb.1906.06064]

34 **Chang CJ**, Lin TL, Tsai YL, Wu TR, Lai WF, Lu CC, Lai HC. Next generation probiotics in disease amelioration. *J Food Drug Anal* 2019; **27**: 615-622 [PMID: 31324278 DOI: 10.1016/j.jfda.2018.12.011]

35 **35 Hu TG**, Wen P, Shen WZ, Liu F, Li Q, Li EN, Liao ST, Wu H, Zou YX. Effect of 1-Deoxynojirimycin Isolated from Mulberry Leaves on Glucose Metabolism and Gut Microbiota in a Streptozotocin-Induced Diabetic Mouse Model. *J Nat Prod* 2019; **82**: 2189-2200 [PMID: 31393724 DOI: 10.1021/acs.jnatprod.9b00205]

36 **Aziz G**, Zaidi A, Tariq M. Compositional Quality and Possible Gastrointestinal Performance of Marketed Probiotic Supplements. *Probiotics Antimicrob Proteins* 2022; **14**: 288-312 [PMID: 35199309 DOI: 10.1007/s12602-022-09931-7]

37 **Sanders ME**, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 605-616 [PMID: 31296969 DOI: 10.1038/s41575-019-0173-3]

38 **Li HY**, Zhou DD, Gan RY, Huang SY, Zhao CN, Shang A, Xu XY, Li HB. Effects and Mechanisms of Probiotics, Prebiotics, Synbiotics, and Postbiotics on Metabolic Diseases Targeting Gut Microbiota: A Narrative Review. *Nutrients* 2021; **13** [PMID: 34579087 DOI: 10.3390/nu13093211]

39 **Mañé J**, Lorén V, Pedrosa E, Ojanguren I, Xaus J, Cabré E, Domènech E, Gassull MA. Lactobacillus fermentum CECT 5716 prevents and reverts intestinal damage on TNBS-induced colitis in mice. *Inflamm Bowel Dis* 2009; **15**: 1155-1163 [PMID: 19266568 DOI: 10.1002/ibd.20908]

40 **Feng T**, Wang J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes* 2020; **12**: 1801944 [PMID: 32795116 DOI: 10.1080/19490976.2020.1801944]

41 **Dowarah R**, Verma AK, Agarwal N, Singh P, Singh BR. Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. *PLoS One* 2018; **13**: e0192978 [PMID: 29518093 DOI: 10.1371/journal.pone.0192978]

42 **Sun Z**, Sun X, Li J, Li Z, Hu Q, Li L, Hao X, Song M, Li C. Using probiotics for type 2 diabetes mellitus intervention: Advances, questions, and potential. *Crit Rev Food Sci Nutr* 2020; **60**: 670-683 [PMID: 30632770 DOI: 10.1080/10408398.2018.1547268]

43 **Zheng J**, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int J Syst Evol Microbiol* 2020; **70**: 2782-2858 [PMID: 32293557 DOI: 10.1099/ijsem.0.004107]

44 **Paulino do Nascimento LC**, Lacerda DC, Ferreira DJS, de Souza EL, de Brito Alves JL. Limosilactobacillus fermentum, Current Evidence on the Antioxidant Properties and Opportunities to be Exploited as a Probiotic Microorganism. *Probiotics Antimicrob Proteins* 2022 [PMID: 35467236 DOI: 10.1007/s12602-022-09943-3]

45 **de Luna Freire MO**, do Nascimento LCP, de Oliveira KÁR, de Oliveira AM, Napoleão TH, Lima MDS, Lagranha CJ, de Souza EL, de Brito Alves JL. Effects of a Mixed *Limosilactobacillus fermentum* Formulation with Claimed Probiotic Properties on Cardiometabolic Variables, Biomarkers of Inflammation and Oxidative Stress in Male Rats Fed a High-Fat Diet. *Foods* 2021; **10** [PMID: 34574313 DOI: 10.3390/foods10092202]

46 **Cavalcante RGS**, de Albuquerque TMR, de Luna Freire MO, Ferreira GAH, Carneiro Dos Santos LA, Magnani M, Cruz JC, Braga VA, de Souza EL, de Brito Alves JL. The probiotic Lactobacillus fermentum 296 attenuates cardiometabolic disorders in high fat diet-treated rats. *Nutr Metab Cardiovasc Dis* 2019; **29**: 1408-1417 [PMID: 31640890 DOI: 10.1016/j.numecd.2019.08.003]

47 **Adeshirlarijaney A**, Gewirtz AT. Considering gut microbiota in treatment of type 2 diabetes mellitus. *Gut Microbes* 2020; **11**: 253-264 [PMID: 32005089 DOI: 10.1080/19490976.2020.1717719]

48 **Archer AC**, Muthukumar SP, Halami PM. Lactobacillus fermentum MCC2759 and MCC2760 Alleviate Inflammation and Intestinal Function in High-Fat Diet-Fed and Streptozotocin-Induced Diabetic Rats. *Probiotics Antimicrob Proteins* 2021; **13**: 1068-1080 [PMID: 33575913 DOI: 10.1007/s12602-021-09744-0]

49 **Balakumar M**, Prabhu D, Sathishkumar C, Prabu P, Rokana N, Kumar R, Raghavan S, Soundarajan A, Grover S, Batish VK, Mohan V, Balasubramanyam M. Improvement in glucose tolerance and insulin sensitivity by probiotic strains of Indian gut origin in high-fat diet-fed C57BL/6J mice. *Eur J Nutr* 2018; **57**: 279-295 [PMID: 27757592 DOI: 10.1007/s00394-016-1317-7]

50 **Hartajanie L**, Fatimah-Muis S, Heri-Nugroho Hs K, Riwanto I, Sulchan M. Probiotics Fermented Bitter Melon Juice as Promising Complementary Agent for Diabetes Type 2: Study on Animal Model. *J Nutr Metab* 2020; **2020**: 6369873 [PMID: 32190386 DOI: 10.1155/2020/6369873]

51 **Chaiyasut C**, Woraharn S, Sivamaruthi BS, Lailerd N, Kesika P, Peerajan S. Lactobacillus fermentum HP3-Mediated Fermented Hericium erinaceus Juice as a Health Promoting Food Supplement to Manage Diabetes Mellitus. *J Evid Based Integr Med* 2018; **23**: 2515690X18765699 [PMID: 29619846 DOI: 10.1177/2515690X18765699]

52 **Guilbaud A**, Howsam M, Niquet-Léridon C, Delguste F, Fremont M, Lestavel S, Maboudou P, Garat A, Schraen S, Onraed B, Foligné B, Boulanger É, Tessier FJ. The Effect of Lactobacillus fermentum ME-3 Treatment on Glycation and Diabetes Complications. *Mol Nutr Food Res* 2020; **64**: e1901018 [PMID: 31991062 DOI: 10.1002/mnfr.201901018]

53 **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]

54 **Yadav R**, Dey DK, Vij R, Meena S, Kapila R, Kapila S. Evaluation of anti-diabetic attributes of Lactobacillus rhamnosus MTCC: 5957, Lactobacillus rhamnosus MTCC: 5897 and Lactobacillus fermentum MTCC: 5898 in streptozotocin induced diabetic rats. *Microb Pathog* 2018; **125**: 454-462 [PMID: 30316007 DOI: 10.1016/j.micpath.2018.10.015]

55 **Yousaf S**, Hussain A, Rehman S, Aslam MS, Abbas Z. Hypoglycemic and hypolipidemic effects of Lactobacillus fermentum, fruit extracts of Syzygium cumini and Momordica charantia on diabetes induced mice. *Pak J Pharm Sci* 2016; **29**: 1535-1540 [PMID: 27731809]

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MINIREVIEWS

COVID-19 associated diabetes mellitus: A review

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Abstract

A significantly higher rate of new-onset diabetes in many coronavirus disease 2019 (COVID-19) patients is a frequently observed phenomenon. The resultant hyperglycemia is known to influence the clinical outcome, thereby increasing the cost of treatment and stay in hospital. This will also affect the post-hospitalization recuperation. It has been observed that new-onset diabetes in COVID-19 patients is associated with considerable increase in morbidity and may be associated with increased mortality in some cases. This mini-review focuses on the possible causes to understand how COVID-19-related diabetes develops, various associated risk factors, and possible mechanism to understand the natural history of the disease process, clinical outcome, associated morbidities and various treatment options in the mana-gement of post COVID-19 diabetes. A literature search was performed in PubMed and other online database using appropriate keywords. A total of 80 articles were found, among which, 53 of the most relevant were evaluated/analyzed and relevant data were included. The studies show that patients who have had severe acute respiratory syndrome coronavirus 2 infection leading to development of COVID-19 may manifest not only with new-onset diabetes but also worsening of pre-existing diabetes. Cytopathic effect and autoimmune destruction of insulin-secreting pancreatic beta cells, cytokine storm during the active phase of infection causing impaired insulin secretion and resistance, drug-induced hyperglycemia, undetected pre-existing hyperglycemia/diabetic condition, and stress-induced impairment of glucose metabolism are some of the

possible potential mechanisms of COVID-19-associated new-onset diabetes mellitus. Many studies published in recent times have found a significantly higher rate of new-onset diabetes mellitus in many COVID-19 patients. Whether it is an inflammatory or immune-mediated response, direct effect of virus or combination of these is unclear. The resultant hyperglycemia is known to influence the clinical outcome and has been associated with considerable increase in morbidity and increased mortality in some cases.

Key Words: Coronavirus disease 2019; Coronavirus disease 2019 associated diabetes; Coronavirus disease 2019 related diabetes; Hyperglycemia in coronavirus disease 2019 patients; New-onset diabetes; Post-coronavirus disease 2019 diabetes

Core Tip: New-onset diabetes is one of the most important complications in patients recovering from coronavirus disease 2019 (COVID-19). This review is focused on different hypotheses that help with understanding of the disease process and suggest management protocols for COVID-19-associated diabetes mellitus.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a global pandemic by the World Health Organization (WHO) in March 2020. It continues to spread worldwide with about 452201564 confirmed cases and 6029852 deaths to date[1].

The speed with which this deadly virus spreads leaves no place for doubt that, at some time, a significant proportion of the world's population will be affected. Therefore, it is a matter of great concern to study the interaction of COVID-19 with other commonly occurring medical conditions to anticipate and find out how they will interact with each other and to decide a protocol for their management. Laboratory reports in almost all critically ill patients show severe hyperglycemia as a common finding and this is often considered a marker of disease severity[2]. A literature search for studies carried out during the pandemic shows that COVID-19 is associated with hyperglycemia in people with and without known diabetes mellitus. Hence, now there is sufficient evidence to support the fact that SARS-CoV-2 infection causes a diabetogenic state in COVID-19 patients[3,4]. In this mini-review, an attempt has been made to understand how COVID-19 related diabetes develops, its pathogenesis, clinical presentation, outcome and management protocol of new-onset diabetes mellitus in COVID-19 patients.

DIABETOGENIC EFFECT OF SARS-CoV-2 INFECTION IN COVID-19

SARS-CoV-2 infection leading to a diabetogenic state in patients of COVID-19 is now a well-established fact. Different studies carried out in the earlier days of the pandemic support this fact and they report that many patients with SARS-CoV-2 infection were diagnosed with diabetes mellitus after COVID-19. It has been found that many patients presented with diabetic ketoacidosis (DKA) or hyperosmolar hyperglycemic state and required higher units of insulin to normalize the blood sugar levels[4-6].

CAUSES/RISK FACTORS

The severity of hyperglycemic levels in confirmed cases of COVID-19 infection was found to be proportional to the severity of infection. This can be attributed to the involvement of one or more inter-related processes like stress response associated with severe illness, cytokine storm with elevated levels of inflammatory markers like interleukin (IL)-6, tumor necrosis factor (TNF)-α, C-reactive protein (CRP),

lactate dehydrogenase and ferritin. Overdoses of steroids, pancreatic beta-cell damage/destruction resulting in a combined effect of insulin resistance and insufficiency disturbing glucose homeostasis have been reported as important risk factors. Apart from this, increasing age, high body mass index (BMI) and family history of diabetes are independent risk factors[7]. To make the situation worse, strict disciplinary actions taken to break the chain of infection (such as repeated rotatory lockdowns) could also have had an adverse impact such as limited access to clinical care, healthy diet and opportunities to exercise[8].

POSSIBLE POTENTIAL MECHANISMS

COVID-19 due to SARS-CoV-2 infection may manifest not only as new-onset diabetes but also causes worsening of pre-existing diabetes. Considering the evolving nature of the COVID-19 pandemic, it is not yet clearly understood whether SARS-CoV-2 infection causes new-onset diabetes by mechanisms similar to those established in the pathogenesis of type 1 or type 2 diabetes mellitus, or whether this itself is an atypical form of diabetes[9]. Moreover, it has also not been established whether COVID-19 patients remain at higher risk for developing new-onset diabetes or related complications following viral clearance and recovery. The literature reveals detailed discussions regarding the possible potential mechanisms for derangement of glucose metabolism leading to the development of hyperglycemia and new-onset diabetes in COVID-19 patients and these can be broadly attributed to the following factors (Figure 1).

Cytopathic effect causing beta-cell damage

The entry portal for SARS-CoV-2 is angiotensin-converting enzyme (ACE)-2 receptor. Along with respiratory epithelial cells, ACE-2 receptors are also present in the kidneys, gastrointestinal tract and pancreas. Following infection, SARS-CoV-2 replicates in human endocrine and exocrine secretory cells of the pancreas[10]. It has been postulated that this causes the destruction of insulin-secreting pancreatic beta cells, which leads to the development of new-onset diabetes in some patients with COVID-19. This phenomenon can be well correlated with that observed during SARS-CoV-1 infection; thus, giving due credit to this hypothesis[11].

Autoimmune destruction of pancreatic beta cells

Apart from direct virus-induced cytotoxicity over insulin-secreting beta cells of the pancreas, another suggestion is that SARS-CoV-2 can trigger an autoimmune response against pancreatic beta-cell antigens, and it has emerged as one of the most prevalent hypotheses behind the etiopathogenesis of type 1 diabetes. According to this theory, the virus-mediated cytotoxicity toward beta cells leads to sequestration of antigens that in turn cause activation of autoreactive T lymphocytes. The resultant autoimmune response ultimately destroys the remainder of the beta-cell mass, leading to insulin-dependent type 1 diabetes in a few weeks to months after infection[12]. This theory cannot completely explain the pathogenesis of immediate onset of diabetes during the acute phase of COVID-19 infection; however, it may hold true for development of hyperglycemia in some patients and later development of diabetes within weeks to months post-COVID recovery. Further research about this would be helpful to reach a more meaningful conclusion.

Host response to COVID-19

As observed in any acute infectious condition, a profound and nonspecific activation of immune mechanisms also occurs in patients with severe COVID-19, escalating the release of counter-regulatory hormones and proinflammatory cytokines such as IL-6 and TNF-α in the form of cytokine storm. This rampant cytokine storm is known to induce insulin resistance and resultant hyperglycemia[13].

Drug-induced iatrogenic effect

The RECOVERY trial in ICU COVID-19 patients requiring respiratory support prompted WHO to reframe guidelines and recommend the use of corticosteroids to reduce the overall mortality and morbidity in such patients[14]. However, corticosteroids are a double-edged sword. On one side, they improve the clinical course of patients during the cytokine storm and thereby prevent death in patients with COVID-19 pneumonia. On the other side, they are also known to be highly diabetogenic drugs. Hyperglycemia is almost inevitable with the doses prescribed for this indication and some cases present with complications like DKA, especially in patients with previously undiagnosed diabetes or prediabetes[8].

Undetected pre-existing diabetes before infection with SARS-CoV-2

The latest report of Diabetes Atlas from the International Diabetes Federation states that almost 50% of the adult population may have undiagnosed diabetes and bear a lifetime risk of diabetes mellitus[15]. This potential at-risk population is the reason for the hike in incidence of new-onset diabetes after COVID-19. Probable causes for this are recent weight gain, worsening of hyperglycemia due to changes

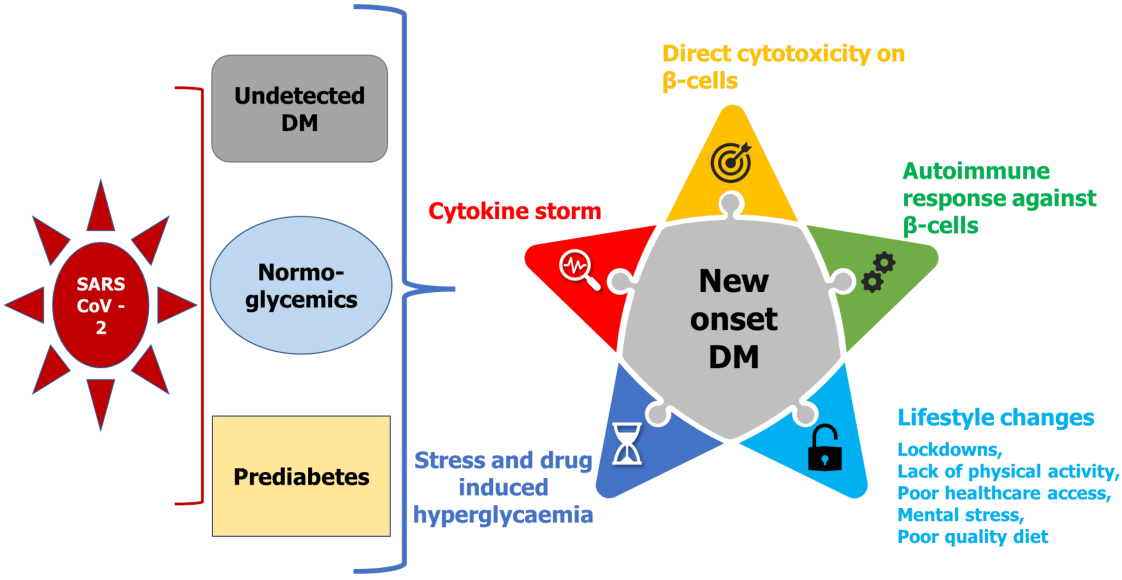

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Figure 1 Possible potential mechanisms for development of post-coronavirus disease 2019 diabetes new onset diabetes. DM: Diabetes mellitus.

in lifestyle such as lack of exercise and reduced physical activity due to lockdown, self-isolation, social distancing and poor diet as a result of lack of access to sufficient quality and quantity of fruits/vegetables during lockdown periods[16].

Acute illness and stress leading to hyperglycemia and new-onset diabetes

Any acute illness and associated stress are the two important common factors that may lead to hyperglycemia in many patients and these patients will form the category of new-onset diabetes mellitus. This was observed during the SARS-CoV-1 pandemic[17]. The cytokine storm due to acute infection by SARS-CoV-2 can cause elevated inflammatory markers like an increase in CRP, erythrocyte sedimentation rate and increased leukocyte count. Cellular stress during acute inflammation causes accelerated lipolysis, thereby increasing the levels of free fatty acids in the circulation, leading to relative insulin deficiency[18].

New-onset diabetes has been reported in most of the earlier studies from different parts of the globe (Table 1)[4,19-30].

CLINICAL OUTCOME AND ASSOCIATED MORBIDITIES

It has been observed that diabetes is the pre-existing condition in most of the patients with COVID-19 disease showing severe morbidity and mortality[31]. The diabetic patients in general were found to have a higher risk of developing diabetic nephropathy, ischemic heart disease, and pneumonia leading to multiorgan failure and acute respiratory distress syndrome (ARDS) as compared to nondiabetic individuals. In addition, diabetic individuals were found to be more prone to ICU admission[32,33]. In subjects with diabetes and COVID-19, the mortality rate ranges from 22% to 31% of all COVID-19 patients[34]. A UK-based study revealed that out of 23 804 deaths in hospitalized COVID-19 patients, 32% had type 2 diabetes and 1.5% had type 1 diabetes mellitus[35].

Obesity, one of the independent risk factors for type 2 diabetes mellitus, is significantly associated with the severity of COVID-19. A cohort study of 2741 hospitalized patients found that among different factors, obesity was strongly associated with COVID-19 hospitalization and risk of critical illness[36]. A retrospective study from Kuwait consisting of 1158 hospitalized COVID-19 patients concluded that patients with morbid obesity needed more ICU admissions [odds ratio (OR), 5.18][37]. A study by Cai *et al*[38] involving 383 hospitalized COVID-19 patients reported that COVID-19 manifestations were more severe in obese patients as compared to patients with normal BMI. They also found an increased OR of developing severe COVID-19 in overweight patients (OR, 1.84; P = 0.05), with the value of odds being higher in obese subjects (OR, 3.40; P = 0.007).

Altered glucose homeostasis and insulin resistance resulting in acute hyperglycemia have been reported during infection in patients hospitalized with viral infections such as human herpes virus 8 and SARS-CoV as a part of normal antiviral responses. Such responses may further increase the risk of developing type 1 or type 2 diabetes mellitus[39]. During the SARS-CoV-1 outbreak in 2003, findings

Table 1 New-onset diabetes studies reported from different parts of globe

Ref.	Country	Study Design	Number of Cases	Results
Li *et al*[4]	China	Retrospective Observational	453	21 % were newly diagnosed with DM
Unsworth *et al* [19]	United Kingdom	Cross-sectional	33 children	30 children with new onset T1D
Ebekozien *et al* [20]	United States	Cross-sectional	64	6 cases with new onset T1D
Armeni *et al* [21]	United Kingdom	Case series	35	5.7 % cases newly presented with DM
Sathish *et al*[22]	China, Italy, United States	Systematic review	3711 cases from 8 studies	492 cases newly presented with DM
Wang *et al*[23]	China	Retrospective	605	176 cases newly detected with DM
Yang *et al*[24]	China	Retrospective Cohort	69	Prevalence: 53.85% in critical cases and 13.95% in moderately severe cases
Fadini *et al*[25]	Italy	Retrospective	413	5 % cases newly detected with DM
Wu *et al*[26]	Australia	Retrospective	8	Newly diagnosed cases showed C-peptide levels, negative anti-GAD antibodies consistent with T2D
Ghosh *et al*[27]	India	Retrospective Cohort	555	Higher levels of FBG, PPBG, HbA1c in newly diagnosed cases
Zhang *et al*[28]	China	Retrospective	312	Higher risk of adverse outcomes
Smith *et al*[29]	United States	Retrospective	184	6 patients showed elevated FBG
Liu *et al*[30]	China	Retrospective	233	Increased risk of in-hospital deaths

DM: Diabetes mellitus; FBG: Fasting blood glucose; GAD: Glutamic acid decarboxylase; HbA1c: Glycated hemoglobin; PPBG: Post-prandial blood glucose; T1D: Type 1 diabetes; T2D: Type 2 diabetes.

from one study involving 39 patients without a history of diabetes mellitus, showed that 20 patients developed diabetes during hospitalization and two of these patients remained diabetic despite receiving 3 years of antidiabetic management during follow-up[11].

In a study by Li *et al*[4], 94 out of 453 COVID-19 patients were diagnosed with new-onset diabetes. These newly diagnosed, post-COVID-19 diabetic patients required admission, intermittent mandatory ventilatory assistance, and demonstrated higher risk of all-cause mortality than those COVID-19 patients who were normoglycemic or had transient hyperglycemia. Also, these COVID-19 patients with pre-existing diabetes and new-onset diabetes demonstrated more severe complications including ARDS, acute renal failure, shock, or hypoalbuminemia as compared to those COVID-19 patients having normal or transiently raised blood sugar levels. Similarly, another multicenter retrospective study by Wang *et al* [23], involving 605 COVID-19 patients found that 29% of patients with newly detected diabetes mellitus experienced a higher rate of in-hospital complications and all-cause mortality as compared to normoglycemic COVID-19 patients over a 28-d period. Finally, another study by Fadini *et al*[25], comprising 413 subjects, reported a significant increase in ICU admissions and a higher percentage of death in patients with new-onset COVID-19-related diabetes compared to COVID-19 patients with pre-existing diabetes or normal blood glucose levels. In a retrospective observational study from Wuhan by Zhang *et al*[40], there was no significant increase in these parameters. Although many other studies have indicated a correlation between new-onset diabetes and COVID-19, experimental findings from several studies like Ibrahim *et al*[41] and Drucker[42] have also reported an inconclusive relationship between the increase in type 1 diabetes mellitus during the COVID-19 pandemic. These observations can be attributed to a lack of strong supporting evidence. Therefore, there is a necessity for further research to elucidate the interconnected relationship between COVID-19-induced diabetes mellitus and associated complications.

MANAGEMENT OF COVID-19-ASSOCIATED DIABETES MELLITUS

Since the explicit mechanisms and epidemiological factors associated with the development of new-onset diabetes following COVID-19 are unknown, it is difficult to frame treatment guidelines for such patients. However, in the light of increasing morbidity and mortality in people with newly diagnosed diabetes mellitus or those with hyperglycemia during admission, treatment protocols should prioritize

the management of acute hyperglycemia. It is also indispensable to diagnose COVID-19-associated diabetes mellitus and manage its metabolic complications such as DKA in patients admitted to the hospital for better clinical outcomes. Insulin requirement is invariably higher in such patients when compared to that in patients with acute illness due to other reasons or non-COVID-19-related DKA[26,43,44]. The exact duration of hospital stay of patients with newly detected diabetes mellitus following SARS-CoV-2 infection cannot be defined. There is a paucity of data in the literature regarding the long-term follow-up of these patients. Patients with stress-induced hyperglycemia may revert to a normoglycemic state once they have recovered from the phase of acute illness. Our experience also shows that most patients who have developed new-onset diabetes following SARS-CoV-2 infection have been found to revert to normoglycemic state within 2–4 wk after recovery, especially patients aged < 60 years. These patients, therefore, may not be labeled as having full-blown diabetes requiring prolonged antidiabetic medication. However, these cases are at high risk for developing diabetes in the future; therefore, they require long-term follow-up to determine a further course of action.

Recently, in a study report from India by Kuchay *et al*[45], three COVID-19 patients presented with acute-onset diabetes mellitus with DKA and had favorable initial response to treatment with intravenous fluids and insulin. Later, these patients were managed with multiple doses of subcutaneous insulin, and after 4–6 wk, they were shifted from insulin to oral hypoglycemic agents. Glutamic acid decarboxylase antibodies were measured in two patients who had tested negative, suggesting a transient insulinopenia in these patients.

Considering associated comorbidities like obesity, hypertension, hypercholesterolemia, coronary artery disease, renal disease, *etc.* in COVID-19 patients, hypoglycemic agents that improve metabolic function without weight gain should be the preferred choice for long-term management in patients following acute SARS-CoV-2 infection and sustained symptoms (*i.e.*, long COVID). Sodium–glucose co-transporter-2 (SGLT2) inhibitors and glucagon-like peptide-1 receptor agonists (GLP-1RAs) are the preferred novel therapeutic options that have a beneficial effect on factors like body weight, glycemic control, and cardiovascular and renal outcomes by reducing the duration of stay, overall morbidity and mortality from cardiac and noncardiac causes[46].

Therapeutic trials

DARE-19: This was a randomized, double-blind, placebo-controlled trial undertaken to study organ-protective effects of dapagliflozin, an SGLT-2 inhibitor. The study was conducted in hospitalized COVID-19 patients with at least one cardiometabolic risk factor (*i.e.*, hypertension, type 2 diabetes, coronary artery disease or chronic kidney disease). The trial excluded critically ill patients. The results showed that although the drug was well-tolerated by patients, it did not have an organ-protective effect. There was no significant improvement in clinical recovery within 30 d of starting the medication[47].

Ongoing trials: Various trials with dipeptidyl peptidase-4 inhibitors, pioglitazone, and the GLP-1RA semaglutide have been designed[48-53], but only a few are currently functional in the recruiting phase [52,53].

Long-term surveillance of COVID-19-associated newly diagnosed diabetes patients is necessary to control their risk factors and achieve adequate glycemic control. Patients with stress hyperglycemia during acute critical illness are at high risk of developing diabetes in the future. Meticulous tracking of such cases for early diagnosis, interventions, and long-term follow-up is necessary. Screening for diabetes in every COVID-19 patient would identify a significant number of cases and the cost-effectiveness of the screening would then need consideration. However, screening for diabetes is advisable at least for high-risk patients because if identified, appropriate management of these cases can be instituted. Also, COVID-19 patients with one or more comorbidities should undergo regular monitoring for cardiac and renal risk factors as well as micro/macrovascular complications.

CONCLUSION

The results of most of the earlier studies show that a significantly higher rate of new-onset diabetes in many COVID-19 patients is a frequently observed phenomenon. The resultant hyperglycemia is known to influence the clinical outcome and has been associated with considerable increase in morbidity and increased mortality in some cases. These issues increase the overall cost of treatment and the length of stay in hospital.

Hyperglycemia may return to normal glycemia in prediabetic or nondiabetic patients once they recover from acute illness and may not require antidiabetic medications. However, long-term follow-up is the key in such cases. Important prognostic factors include early diagnosis, associated other comorbidities, interventions, and longer surveillance of patients with stress hyperglycemia and/or new-onset diabetes so that we can ensure that their risk factors are managed and good glycemic control is achieved.

Studies published in recent times assessed the findings of hospitalized COVID-19 patients. There are no or limited data available from patients who were asymptomatic or had mild disease managed in community COVID care centers or in home isolation. So, there is likely to be a greater number of cases

of newly detected diabetes in COVID-19 patients worldwide. Hence, a large population of patients needs to be followed up globally to have better understanding of this phenomenon, involving an epidemiological and interventional approach.

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REFERENCES

1 **WHO**. WHO coronavirus disease (COVID-19) dashboard. [cited 14 March 2022]. Available from: https://covid19.who.int/
2 **Jivanji CJ**, Asrani VM, Windsor JA, Petrov MS. New-Onset Diabetes After Acute and Critical Illness: A Systematic Review. *Mayo Clin Proc* 2017; **92**: 762-773 [PMID: 28302323 DOI: 10.1016/j.mayocp.2016.12.020]
3 **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]
4 **Li H**, Tian S, Chen T, Cui Z, Shi N, Zhong X, Qiu K, Zhang J, Zeng T, Chen L, Zheng J. Newly diagnosed diabetes is associated with a higher risk of mortality than known diabetes in hospitalized patients with COVID-19. *Diabetes Obes Metab* 2020; **22**: 1897-1906 [PMID: 32469464 DOI: 10.1111/dom.14099]
5 **Chee YJ**, Ng SJH, Yeoh E. Diabetic ketoacidosis precipitated by Covid-19 in a patient with newly diagnosed diabetes mellitus. *Diabetes Res Clin Pract* 2020; **164**: 108166 [PMID: 32339533 DOI: 10.1016/j.diabres.2020.108166]
6 **Ren H**, Yang Y, Wang F, Yan Y, Shi X, Dong K, Yu X, Zhang S. Association of the insulin resistance marker TyG index with the severity and mortality of COVID-19. *Cardiovasc Diabetol* 2020; **19**: 58 [PMID: 32393351 DOI: 10.1186/s12933-020-01035-2]
7 **Farag AA**, Hassanin HM, Soliman HH, Sallam A, Sediq AM, Abd Elbaser ES, Elbanna K. Newly Diagnosed Diabetes in Patients with COVID-19: Different Types and Short-Term Outcomes. *Trop Med Infect Dis* 2021; **6** [PMID: 34449740 DOI: 10.3390/tropicalmed6030142]
8 **Unnikrishnan R**, Misra A. Diabetes and COVID19: a bidirectional relationship. *Nutr Diabetes* 2021; **11**: 21 [PMID: 34168110 DOI: 10.1038/s41387-021-00163-2]
9 **Metwally AA**, Mehta P, Johnson BS, Nagarjuna A, Snyder MP. COVID-19-Induced New-Onset Diabetes: Trends and Technologies. *Diabetes* 2021; **70**: 2733-2744 [PMID: 34686519 DOI: 10.2337/dbi21-0029]
10 **Müller JA**, Groß R, Conzelmann C, Krüger J, Merle U, Steinhart J, Weil T, Koepke L, Bozzo CP, Read C, Fois G, Eiseler T, Gehrmann J, van Vuuren J, Wessbecher IM, Frick M, Costa IG, Breunig M, Grüner B, Peters L, Schuster M, Liebau S, Seufferlein T, Stenger S, Stenzinger A, MacDonald PE, Kirchhoff F, Sparrer KMJ, Walther P, Lickert H, Barth TFE, Wagner M, Münch J, Heller S, Kleger A. SARS-CoV-2 infects and replicates in cells of the human endocrine and exocrine pancreas. *Nat Metab* 2021; **3**: 149-165 [PMID: 33536639 DOI: 10.1038/s42255-021-00347-1]
11 **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]

12 **Boddu SK**, Aurangabadkar G, Kuchay MS. New onset diabetes, type 1 diabetes and COVID-19. *Diabetes Metab Syndr* 2020; **14**: 2211-2217 [PMID: 33395782 DOI: 10.1016/j.dsx.2020.11.012]

13 **Papachristou S**, Stamatiou I, Stoian AP, Papanas N. New-Onset Diabetes in COVID-19: Time to Frame Its Fearful Symmetry. *Diabetes Ther* 2021; **12**: 461-464 [PMID: 33367980 DOI: 10.1007/s13300-020-00988-7]

14 **RECOVERY Collaborative Group**, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021; **384**: 693-704 [PMID: 32678530 DOI: 10.1056/NEJMoa2021436]

15 Diabetes now affects one in 10 adults worldwide. Nov 2, 2021 [cited 14 March 2022]. Available from: https://www.idf.org/news/240:diabetes-now-affects-one-in-10-adults-worldwide.html

16 **Deschasaux-Tanguy M**, Druesne-Pecollo N, Esseddik Y, de Edelenyi FS, Allès B, Andreeva VA, Baudry J, Charreire H, Deschamps V, Egnell M, Fezeu LK, Galan P, Julia C, Kesse-Guyot E, Latino-Martel P, Oppert JM, Péneau S, Verdot C, Hercberg S, Touvier M. Diet and physical activity during the coronavirus disease 2019 (COVID-19) lockdown (March-May 2020): results from the French NutriNet-Santé cohort study. *Am J Clin Nutr* 2021; **113**: 924-938 [PMID: 33675635 DOI: 10.1093/ajcn/nqaa336]

17 **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]

18 **Capes SE**, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. *Lancet* 2000; **355**: 773-778 [PMID: 10711923 DOI: 10.1016/S0140-6736(99)08415-9]

19 **Unsworth R**, Wallace S, Oliver NS, Yeung S, Kshirsagar A, Naidu H, Kwong RMW, Kumar P, Logan KM. New-Onset Type 1 Diabetes in Children During COVID-19: Multicenter Regional Findings in the U.K. *Diabetes Care* 2020; **43**: e170-e171 [PMID: 32816997 DOI: 10.2337/dc20-1551]

20 **Ebekozien OA**, Noor N, Gallagher MP, Alonso GT. Type 1 Diabetes and COVID-19: Preliminary Findings From a Multicenter Surveillance Study in the U.S. *Diabetes Care* 2020; **43**: e83-e85 [PMID: 32503837 DOI: 10.2337/dc20-1088]

21 **Armeni E**, Aziz U, Qamar S, Nasir S, Nethaji C, Negus R, Murch N, Beynon HC, Bouloux P, Rosenthal M, Khan S, Yousseif A, Menon R, Karra E. Protracted ketonaemia in hyperglycaemic emergencies in COVID-19: a retrospective case series. *Lancet Diabetes Endocrinol* 2020; **8**: 660-663 [PMID: 32621809 DOI: 10.1016/S2213-8587(20)30221-7]

22 **Sathish T**, Kapoor N, Cao Y, Tapp RJ, Zimmet P. Proportion of newly diagnosed diabetes in COVID-19 patients: A systematic review and meta-analysis. *Diabetes Obes Metab* 2021; **23**: 870-874 [PMID: 33245182 DOI: 10.1111/dom.14269]

23 **Wang S**, Ma P, Zhang S, Song S, Wang Z, Ma Y, Xu J, Wu F, Duan L, Yin Z, Luo H, Xiong N, Xu M, Zeng T, Jin Y. Fasting blood glucose at admission is an independent predictor for 28-day mortality in patients with COVID-19 without previous diagnosis of diabetes: a multi-centre retrospective study. *Diabetologia* 2020; **63**: 2102-2111 [PMID: 32647915 DOI: 10.1007/s00125-020-05209-1]

24 **Yang J-K**, Jin J-M, Liu S, Bai P, He W, Wu F, Liu X-F, Chai Z-L, Han D-M. New onset COVID-19–related diabetes: an indicator of mortality. 2020 Preprint. Available from: medRxiv: 2020.04.08.20058040 [DOI: 10.1101/2020.04.08.20058040]

25 **Fadini GP**, Morieri ML, Boscari F, Fioretto P, Maran A, Busetto L, Bonora BM, Selmin E, Arcidiacono G, Pinelli S, Farnia F, Falaguasta D, Russo L, Voltan G, Mazzocut S, Costantini G, Ghirardini F, Tresso S, Cattelan AM, Vianello A, Avogaro A, Vettor R. Newly-diagnosed diabetes and admission hyperglycemia predict COVID-19 severity by aggravating respiratory deterioration. *Diabetes Res Clin Pract* 2020; **168**: 108374 [PMID: 32805345 DOI: 10.1016/j.diabres.2020.108374]

26 **Wu L**, Girgis CM, Cheung NW. COVID-19 and diabetes: Insulin requirements parallel illness severity in critically unwell patients. *Clin Endocrinol (Oxf)* 2020; **93**: 390-393 [PMID: 32683745 DOI: 10.1111/cen.14288]

27 **Ghosh A**, Anjana RM, Shanthi Rani CS, Jeba Rani S, Gupta R, Jha A, Gupta V, Kuchay MS, Luthra A, Durrani S, Dutta K, Tyagi K, Unnikrishnan R, Srivastava BK, Ramu M, Sastry NG, Gupta PK, Umasankari G, Jayashri R, Mohan V, Misra A. Glycemic parameters in patients with new-onset diabetes during COVID-19 pandemic are more severe than in patients with new-onset diabetes before the pandemic: NOD COVID India Study. *Diabetes Metab Syndr* 2021; **15**: 215-220 [PMID: 33450530 DOI: 10.1016/j.dsx.2020.12.033]

28 **Zhang J**, Kong W, Xia P, Xu Y, Li L, Li Q, Yang L, Wei Q, Wang H, Li H, Zheng J, Sun H, Xia W, Liu G, Zhong X, Qiu K, Li Y, Wang Y, Song X, Liu H, Xiong S, Liu Y, Cui Z, Hu Y, Chen L, Pan A, Zeng T. Impaired Fasting Glucose and Diabetes Are Related to Higher Risks of Complications and Mortality Among Patients With Coronavirus Disease 2019. *Front Endocrinol (Lausanne)* 2020; **11**: 525 [PMID: 32754119 DOI: 10.3389/fendo.2020.00525]

29 **Smith SM**, Boppana A, Traupman JA, Unson E, Maddock DA, Chao K, Dobesh DP, Brufsky A, Connor RI. Impaired glucose metabolism in patients with diabetes, prediabetes, and obesity is associated with severe COVID-19. *J Med Virol* 2021; **93**: 409-415 [PMID: 32589756 DOI: 10.1002/jmv.26227]

30 **Liu Y**, Lu R, Wang J, Cheng Q, Zhang R, Zhang S, Le Y, Wang H, Xiao W, Gao H, Zeng L, Hong T. Diabetes, even newly defined by HbA1c testing, is associated with an increased risk of in-hospital death in adults with COVID-19. *BMC Endocr Disord* 2021; **21**: 56 [PMID: 33771154 DOI: 10.1186/s12902-021-00717-6]

31 **Barbu MG**, Thompson RJ, Thompson DC, Cretoiu D, Suciu N. The Impact of SARS-CoV-2 on the Most Common Comorbidities-A Retrospective Study on 814 COVID-19 Deaths in Romania. *Front Med (Lausanne)* 2020; **7**: 567199 [PMID: 33015111 DOI: 10.3389/fmed.2020.567199]

32 **Erener S**. Diabetes, infection risk and COVID-19. *Mol Metab* 2020; **39**: 101044 [PMID: 32585364 DOI: 10.1016/j.molmet.2020.101044]

33 **Selvin E**, Juraschek SP. Diabetes Epidemiology in the COVID-19 Pandemic. *Diabetes Care* 2020; **43**: 1690-1694 [PMID: 32540920 DOI: 10.2337/dc20-1295]

34 **Singh AK**, Gupta R, Ghosh A, Misra A. Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. *Diabetes Metab Syndr* 2020; **14**: 303-310 [PMID: 32298981 DOI: 10.1016/j.dsx.2020.04.004]

35 **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]

36 **Petrilli CM**, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, Tobin KA, Cerfolio RJ, Francois F, Horwitz LI. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ* 2020; **369**: m1966 [PMID: 32444366 DOI: 10.1136/bmj.m1966]

37 **Al-Sabah S**, Al-Haddad M, Al-Youha S, Jamal M, Almazeedi S. COVID-19: Impact of obesity and diabetes on disease severity. *Clin Obes* 2020; **10**: e12414 [PMID: 33079448 DOI: 10.1111/cob.12414]

38 **Cai Q**, Chen F, Wang T, Luo F, Liu X, Wu Q, He Q, Wang Z, Liu Y, Liu L, Chen J, Xu L. Obesity and COVID-19 Severity in a Designated Hospital in Shenzhen, China. *Diabetes Care* 2020; **43**: 1392-1398 [PMID: 32409502 DOI: 10.2337/dc20-0576]

39 **Sobngwi E**, Choukem SP, Agbalika F, Blondeau B, Fetita LS, Lebbe C, Thiam D, Cattan P, Larghero J, Foufelle F, Ferre P, Vexiau P, Calvo F, Gautier JF. Ketosis-prone type 2 diabetes mellitus and human herpesvirus 8 infection in sub-saharan africans. *JAMA* 2008; **299**: 2770-2776 [PMID: 18560004 DOI: 10.1001/jama.299.23.2770]

40 **Zhang Y**, Li H, Zhang J, Cao Y, Zhao X, Yu N, Gao Y, Ma J, Zhang H, Guo X, Liu X. The clinical characteristics and outcomes of patients with diabetes and secondary hyperglycaemia with coronavirus disease 2019: A single-centre, retrospective, observational study in Wuhan. *Diabetes Obes Metab* 2020; **22**: 1443-1454 [PMID: 32406594 DOI: 10.1111/dom.14086]

41 **Ibrahim S**, Monaco GSF, Sims EK. Not so sweet and simple: impacts of SARS-CoV-2 on the β cell. *Islets* 2021; **13**: 66-79 [PMID: 33970787 DOI: 10.1080/19382014.2021.1909970]

42 **Drucker DJ**. Diabetes, obesity, metabolism, and SARS-CoV-2 infection: the end of the beginning. *Cell Metab* 2021; **33**: 479-498 [PMID: 33529600 DOI: 10.1016/j.cmet.2021.01.016]

43 **Coppelli A**, Giannarelli R, Aragona M, Penno G, Falcone M, Tiseo G, Ghiadoni L, Barbieri G, Monzani F, Virdis A, Menichetti F, Del Prato S; Pisa COVID-19 Study Group. Hyperglycemia at Hospital Admission Is Associated With Severity of the Prognosis in Patients Hospitalized for COVID-19: The Pisa COVID-19 Study. *Diabetes Care* 2020; **43**: 2345-2348 [PMID: 32788285 DOI: 10.2337/dc20-1380]

44 **Bornstein SR**, Rubino F, Khunti K, Mingrone G, Hopkins D, Birkenfeld AL, Boehm B, Amiel S, Holt RI, Skyler JS, DeVries JH, Renard E, Eckel RH, Zimmet P, Alberti KG, Vidal J, Geloneze B, Chan JC, Ji L, Ludwig B. Practical recommendations for the management of diabetes in patients with COVID-19. *Lancet Diabetes Endocrinol* 2020; **8**: 546-550 [PMID: 32334646 DOI: 10.1016/S2213-8587(20)30152-2]

45 **Kuchay MS**, Reddy PK, Gagneja S, Mathew A, Mishra SK. Short term follow-up of patients presenting with acute onset diabetes and diabetic ketoacidosis during an episode of COVID-19. *Diabetes Metab Syndr* 2020; **14**: 2039-2041 [PMID: 33113470 DOI: 10.1016/j.dsx.2020.10.015]

46 **Zelniker TA**, Wiviott SD, Raz I, Im K, Goodrich EL, Furtado RHM, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Sabatine MS. Comparison of the Effects of Glucagon-Like Peptide Receptor Agonists and Sodium-Glucose Cotransporter 2 Inhibitors for Prevention of Major Adverse Cardiovascular and Renal Outcomes in Type 2 Diabetes Mellitus. *Circulation* 2019; **139**: 2022-2031 [PMID: 30786725 DOI: 10.1161/CIRCULATIONAHA.118.038868]

47 **Kosiborod MN**, Esterline R, Furtado RHM, Oscarsson J, Gasparyan SB, Koch GG, Martinez F, Mukhtar O, Verma S, Chopra V, Buenconsejo J, Langkilde AM, Ambery P, Tang F, Gosch K, Windsor SL, Akin EE, Soares RVP, Moia DDF, Aboudara M, Hoffmann Filho CR, Feitosa ADM, Fonseca A, Garla V, Gordon RA, Javaheri A, Jaeger CP, Leaes PE, Nassif M, Pursley M, Silveira FS, Barroso WKS, Lazcano Soto JR, Nigro Maia L, Berwanger O. Dapagliflozin in patients with cardiometabolic risk factors hospitalised with COVID-19 (DARE-19): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Diabetes Endocrinol* 2021; **9**: 586-594 [PMID: 34302745 DOI: 10.1016/S2213-8587(21)00180-7]

48 Effects of DPP4 inhibition on COVID-19. [Accessed 2022 Feb 28]. In: ClinicalTrials.gov. Bethesda (MD): U.S. National Library of Medicine. Available from https://clinicaltrials.gov/ct2/show/NCT04341935 ClinicalTrials.gov identifier NCT04341935

49 Efficacy and safety of dipeptidyl peptidase-4 inhibitors in diabetic patients with established COVID-19. [Accessed 2022 Feb 28]. In: ClinicalTrials.gov. Bethesda (MD): U.S. National Library of Medicine. Available from https://clinicaltrials.gov/ct2/show/NCT04371978 ClinicalTrials.gov identifier NCT04371978

50 The effect of sitagliptin treatment in COVID-19 positive diabetic patients (SIDIACO). [Accessed 2022 Feb 28]. In: ClinicalTrials.gov. Bethesda (MD): U.S. National Library of Medicine. Available from https://clinicaltrials.gov/ct2/show/NCT04365517 ClinicalTrials.gov identifier NCT04365517

51 Metformin glycinate in patients with MS or DM2, hospitalized with COVID-19 and SARS secondary to SARS-CoV-2 (DMMETCOV19). [Accessed 2022 Feb 28]. In: ClinicalTrials.gov. Bethesda (MD): U.S. National Library of Medicine. Available from https://clinicaltrials.gov/ct2/show/NCT04626089 ClinicalTrials.gov identifier NCT04626089

52 Effect of pioglitazone on T2DM patients with COVID-19 (PIOQ8). [Accessed 2022 Feb 28]. In: ClinicalTrials.gov. Bethesda (MD): U.S. National Library of Medicine. Available from https://clinicaltrials.gov/ct2/show/NCT04604223 ClinicalTrials.gov identifier NCT04604223

53 Semaglutide to reduce myocardial injury in patients with COVID-19 (SEMPATICO). [Accessed 2022 Feb 28]. In: ClinicalTrials.gov. Bethesda (MD): U.S. National Library of Medicine. Available from https://clinicaltrials.gov/ct2/show/NCT04615871 ClinicalTrials.gov identifier NCT04615871

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MINIREVIEWS

Effectiveness and safety of COVID-19 vaccines in patients with diabetes as a factor for vaccine hesitancy

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Abstract

Diabetes mellitus is one of the most common comorbid conditions encountered in patients with severe acute respiratory syndrome coronavirus 2 infection accompanied by significantly increased mortality, prolonged hospital stay, and requirement of invasive mechanical ventilation. This review aims to present the effectiveness and safety profile of available coronavirus disease 2019 (COVID-19) vaccines in people with diabetes as a potential cause of hesitancy for vaccination. Data from published research proves a robust immune response following immunization for COVID-19 in diabetic patients with substantial production of virus-neutralizing antibodies; however, the observed immune response was unequivocally weaker than that in individuals without diabetes. This observation was further enhanced by the findings that worse glycemic control was associated with more suppressed antibody production. In contrast, individuals with optimal glycemic control performed similarly to healthy controls. In addition to the need for strict glucose monitoring and adequate diabetes treatment, those findings

reinforce the concept of diabetes-induced secondary immune deficiency and necessitate the application of booster doses to diabetic patients with priority. Nevertheless, after vaccination, reported adverse events were not different from those in the general population. No increase in severe adverse events was documented. While single case reports detected transient increases in blood glucose post-vaccination, more extensive trials could not replicate such a relationship.

Key Words: COVID-19; COVID-19 vaccines; Diabetes; Vaccine effectiveness; Vaccine; Vaccine hesitancy

Core Tip: Diabetes mellitus is a crucial contributor to coronavirus disease 2019 (COVID-19). This review highlights published research on the effectiveness of vaccination against COVID-19 and related adverse events. Despite data of a notable decrease in the immune response to vaccination of diabetic patients, studies point out the importance of strict glycemic control to achieve adequate immunity against severe acute respiratory syndrome coronavirus 2 and the need to prioritize people with diabetes for the administration of booster doses. Regarding adverse events, none were increased in frequency in the diabetic population, except sporadic transient hyperglycemia observed post-vaccination.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the novel coronavirus causing coronavirus disease 2019 (COVID-19) encountered since December 8, 2019 when the first cases of pneumonia of unknown origin etiology were described in Wuhan, Hubei Province, China[1]. Since then, the World Health Organization has reported more than 472 million confirmed cases of SARS-CoV-2 infection and more than 6 million deaths[2]. Closely related to SARS-CoV-2, the SARS-CoV pathogen causing a pandemic in 2002-2003 was also established to have originated in bats that probably serve as the natural reservoir host for those two viruses[3]. Another related betacoronavirus, the Middle East respiratory syndrome virus, is the cause of frequently emerging local outbreaks. It was first hypothesized that the Middle East respiratory syndrome coronavirus originated in bats. Still, it was then unanimously proved that the reservoir host was dromedary camels causing spillovers to humans[4]. Zoonotic transmission of novel coronaviruses to humans has been suggested to continue as more and more viruses are detected in bats and spill over to humans[5]. The fatality risk has been estimated to be 0.5% to 1.3% of all confirmed COVID-19 cases, with significantly higher rates in advanced age groups, reaching 18.4% in hospitalized patients over 80 years old[6,7].

COVID-19 AND DIABETES PATIENTS

Diabetes mellitus is a chronic metabolic disease characterized by impaired glucose metabolism and increased blood sugar levels as a result of absolute insulin deficiency due to autoimmune destruction of the pancreatic beta-cells (type 1 diabetes and latent autoimmune diabetes of adulthood) or impaired insulin action and a progressive loss of adequate β-cell insulin secretion due to insulin resistance in the target tissues (type 2 diabetes)[8]. Besides increased blood glucose levels, diabetes is associated with numerous chronic microvascular and macrovascular complications, determining diabetes as a cardiovascular disease[8]. Moreover, diabetes is associated with chronic low-grade inflammation[9] and an increased risk of thrombotic events[10], dyslipidemia[11], and metabolic syndrome[12]. Finally, it is a common disease that affects approximately 537 million adults. More than 240 million adults live with undiagnosed diabetes[13]. Collectively, these facts are a prerequisite for determining diabetes as a major risk factor for COVID-19[14]. It is considered that diabetic patients are a high-risk population with a calculated six-times greater risk for hospitalization and twelve-times greater risk for death than the healthy population[15]. Therefore, guidelines clearly state that patients with diabetes are strongly recommended to be vaccinated against COVID-19[16].

It is now widely accepted that diabetes, ischemic heart disease, hypertension, and cerebrovascular disease are the most common chronic comorbid conditions in people suffering from severe COVID-19

and requiring intensive care unit admission[17]. Many studies suggest that prolonged hyperglycemia is related to the increased frequency and severity of any infection, not just COVID-19. A matched cohort study among English primary care patients found that 6% of infection-related hospital admissions and 12% of SARS-CoV-2-related deaths were attributable to diabetes. The incidence rate ratios were highest for soft tissue, bone, and joint infections and sepsis[18].

A retrospective observational study of clinical outcomes in 1122 confirmed cases of COVID-19 found that the mortality rate in patients with diabetes and/or uncontrolled hyperglycemia was 28.8%, significantly higher compared to 6.2% in patients without diabetes or hyperglycemia. Furthermore, the median length of stay was also longer among discharged survivors in diabetic or hyperglycemic patients. Both findings imply the need for meticulous hyperglycemia management in hospitalized COVID-19 patients[19].

Both type 1 and type 2 diabetes mellitus were associated with worse rates of all-cause mortality owing to the COVID-19 pandemic in a large population-based cohort study encompassing more than 98% of primary care practice patients in England[20]. It identified a steep and sizable relationship between HbA1c values and death outcomes. Compared to individuals with optimal HbA1c values (6.5%-7.0%), those with HbA1c > 10% had a dramatically increased in-hospital all-cause mortality (hazard ratio: 2.23; 95% confidence interval: 1.50–3.30; $P < 0.0001$ in type 1 diabetes and hazard ratio: 1.61; 95% confidence interval: 1.47–1.77; $P < 0.0001$ in type 2 diabetes), independent from other risk factors[20].

Obesity, being strongly associated with diabetes mellitus type 2, has been investigated as a critical factor in the immune dysregulation that accompanies severe COVID-19. For example, in a French center, the relative risk for the need for invasive mechanical ventilation was seven-fold greater in patients with a body mass index > 30 kg/m^2 than those within the normal range of body mass index[21].

Similarly, it has been discovered that obesity alters the immune response to influenza infection and vaccination. Compared to vaccinated normal-weight individuals, vaccinated obese adults demonstrated double the relative risk for influenza or influenza-like infection, despite evidence of seroconversion. The hyperleptinemia and hyperinsulinemia accompanying the obese state contribute to T cell dysfunction, leading to impaired immune response[22].

Monocytes and macrophages appear to have a hallmark role in the dysregulated immune state of severe COVID-19 infections. Experimental data show that higher extracellular glucose concentrations promote sustained monocyte glycolysis, increased SARS-CoV-2 replication in antigen-presenting cells, and proinflammatory cytokine expression, leading to T-cell dysfunction and lung epithelial damage [23]. Therefore, it has been hypothesized that mitochondrial reactive oxygen species generation stimulated by SARS-CoV-2 leads to hypoxia-inducible factor alpha synthesis. Hypoxia-inducible factor alpha increased the expression of ACE2 (the entry point of SARS-CoV-2 into lung epithelial cells), interleukin-1β, tumor necrosis factor-α, interleukin-6, and interferons α, β, and λ in infected monocytes. Those findings suggest that hypoxia-inducible factor alpha is necessary to induce glycolysis and the consequent proinflammatory state of SARS-CoV-2-infected monocytes[23].

In addition to proinflammatory cytokines and coagulation factor modulation, SARS-CoV-2–induced reactive oxygen species production and viral activation of the renin-angiotensin system (leading to increased angiotensin II expression) lead to insulin resistance, hyperglycemia, and vascular endothelial injury, contributing to acute respiratory distress syndrome as well as cardiovascular, cerebrovascular thromboembolic complications, and disseminated intravascular coagulation[24].

ROUTINE VACCINES FOR DIABETES PATIENTS

Diabetes patients (both type 1 and type 2) are at an increased risk of significant complications from vaccine-preventable illnesses, including hospitalizations and death. Even properly managed diabetes could be associated with second immune deficiency and increased susceptibility to infections due to impaired cellular immune function. Diabetes patients are more likely to die from pneumonia, bacteremia, and meningitis. In line with this, immunization offers the most effective protection against vaccine-preventable illnesses. Therefore, in the next paragraph, we provide information about the routine and recommended vaccination of diabetes patients to emphasize the solid background behind the vaccines that can be used to improve patient and doctor confidence in the vaccines while decreasing vaccine hesitancy.

Moreover, vaccine side effects are often minor and resolve on their own. Severe adverse effects are quite uncommon. Given all the information above and the usually immunocompromised status of diabetes patients, many routine vaccines are officially recommended. For example, the National Health Service in Great Britain recommends the inactivated intramuscular vaccine against seasonal influenza for patients with diabetes types 1 and 2. This is because the risk of severe disease is higher for them than for people without diabetes[25].

The Centers for Disease Control and Prevention (CDC) gives the same recommendation according to seasonal influenza. All patients with diabetes from 6 mo are recommended for the inactivated intramuscular vaccine against the disease. The CDC does not recommend the live attenuated influenza

vaccine, also known as the nasal spray, for people with diabetes types 1 and 2[26].

A multicenter, randomized, and controlled study from the Republic of Korea demonstrated the safety and effectiveness of trivalent subunit inactivated intramuscular influenza vaccine, which contained an A/California/7/2009 (H1N1)-like strain, an A/Victoria/361/2011 (H3N2)-like strain, and a B/Brisbane/60/2008-like strain[27]. The World Health Organization recommended the strains during the 2012-2013 influenza season. The scientists observed similar results of seroprotection rates against the A/H3N2 and the B strains in the diabetic and non-diabetic groups. However, the diabetic group had significantly lower rates than those in the non-diabetic controls for the A/H1N1 strain. In both groups, 1 mo after vaccination, the geometric mean titers and seroprotection rates had increased dramatically for all three virus strains ($P < 0.001$)[27]. According to this study, 6 mo after vaccination, differences in the immunogenicity profiles between the diabetic and the non-diabetic groups were proven, with the seroprotection rate much lower in the elderly diabetes group than in the elderly control group. The safety of the trivalent subunit inactivated intramuscular influenza vaccine was established during the study, and all the participants confirmed that the vaccine was well tolerated. The post-vaccination reactions were mild to moderate, with tenderness at the injection site being the most frequent local reaction. In the diabetes group, 34.3% of the patients reported this local adverse event compared to 45.3% in the control group ($P < 0.001$). From the systemic reactions, myalgia was most reported, followed by tiredness, headache, malaise, chills, and arthralgia[27].

Another highly recommended vaccine for patients with diabetes is the vaccine against pneumococcal disease. The CDC recommends the pneumococcal vaccine for all children younger than 2 years and all adults 65 years or older. In addition, adults aged 19 through 64 are recommended for vaccination if they have chronic illnesses (including diabetes), human immunodeficiency virus/acquired immunodeficiency syndrome, or cancer or smoke cigarettes[28].

In a randomized controlled trial among elderly adults with comorbidities, the 13-valent pneumococcal conjugate vaccine showed significantly higher vaccine efficacy among subjects with diabetes mellitus[29].

A German retrospective cohort study proved the effectiveness of the 23-valent polysaccharide vaccine for invasive pneumococcal disease provoked by *Streptococcus pneumoniae* 22/23 serotypes. Therefore, scientists have recommended increasing the vaccination coverage of 23-valent polysaccharide vaccine among elderly adults in Germany[30].

Herpes zoster is an infection that occurs after reactivation of the varicella-zoster virus and is most common in people older than 50 years who have age-related fading of the immune function and concomitant comorbidities[31]. Herpes zoster is more prevalent among people with diabetes mellitus [32]. There are currently two vaccines against herpes zoster, a live-attenuated vaccine and a recombinant zoster vaccine. The effectiveness of both vaccines resulted in a significant decrease in the incidence of the disease in the older adult population[33-35].

Regarding immune responses after vaccination, diabetes patients mounted an adequate B-cell immune response after influenza and the 23-valent polysaccharide vaccine[36]. However, they had a lower response to the hepatitis B vaccine[37]. All findings support the notion that vaccines for vaccine-preventable illnesses should be administered in a timely manner to diabetic patients, given that this population are susceptible to infection and have a higher risk of diabetes deterioration during infections.

COVID-19 VACCINES, AUTOIMMUNITY, AND GLUCOSE METABOLISM

Autoimmune inflammatory diseases are characterized by an abnormal immune response to self antigens[38]. The interactions between people with autoimmune diseases and SARS-CoV-2 infection are generally unexpected. The mechanisms underlying the possible complications and fatal outcomes are not fully understood. COVID-19, like other viral infections, has the potential to cause a flare, including in diabetes patients[39,40].

Although preliminary findings revealed that autoimmune diseases did not enhance the incidence of SARS-CoV-2 infection and severe disease[41], autoimmune disorders are associated with organ damage, chronic cardiovascular, metabolic and respiratory comorbidities, susceptibility to bacterial infections, and sometimes, B cell depletion therapy and usage of high-dose glucocorticoids. All these factors may enhance the risk of a poor prognosis for patients during the COVID-19 course[42]. As a result, COVID-19 preventive methods should focus on the unique group of autoimmune disease patients, with immunization against SARS-CoV-2 being one of the most promising approaches. Nonetheless, because of growing findings, their safety and efficacy should be primarily and regularly assessed in different patient populations, including patients with diabetes[43].

We will focus on vaccine hesitancy in patients with diabetes later. Still, aside from the fear of immunization-related autoimmunity, there is considerable evidence that the development of autoimmune diseases is influenced by a variety of other variables. In fact, because autoimmune illnesses can develop without immunizations, it is impossible to conclude that vaccines alone induce autoimmunity[44].

Also, people with autoimmune disorders are most concerned about whether the risk of disease flare or aggravation increases following immunization. However, more than 5000 studies confirmed that those with autoimmune illnesses were not at risk of aggravation or worsening conditions[45].

The approved COVID-19 vaccines have played the most significant role in the battle with the SARS-CoV-2 virus to reduce disease severity and mortality among those affected, especially those with diabetes. As of March 16, 2022, more than 10 billion doses of different COVID-19 vaccines have been administered worldwide, including booster doses[46]. So far, we know that although the COVID-19 vaccinations' immunogenicity and efficacy in the autoimmune disease patient population may be lower than in healthy controls, they are typically comparable. Furthermore, data on the vaccines' effectiveness in the autoimmune disease population of adults and children are lacking since only a few studies follow up on the duration of protection and different modalities of immune responses after immunization[47].

Vaccination is recommended as a priority for people with diabetes. The aim is to elicit a sustained immune response in the target population. There is evidence that glycemic control in diabetes significantly affects the immune response[48]. Therefore, it is important to determine whether glycemic disturbances occur before or after vaccination against COVID-19 in people with diabetes.

Monitoring blood glucose levels became critical during the COVID-19 pandemic because the data show two to three times higher hospitalizations and double the mortality rate among patients with simultaneous diabetes and COVID-19[49-51]. It also turned out that emerging diabetes, hyperosmolar hyperglycemic syndrome, and diabetic ketoacidosis could accompany post-COVID syndrome[52,53].

Very few studies have been conducted on how vaccination affects blood sugar levels. However, some effects of COVID-19 on glycolytic metabolism are already known[54]. Several cases of hyperglycemia followed by vaccination against COVID-19 were reported[53,55,56]. One diabetic woman and two diabetic men had post-vaccine hyperglycemia within 6 d of receiving the first dose of the Covishield vaccine (AstraZeneca). Hyperglycemia passed after about a month in the woman after treatment with a higher dose of metformin. At the same time, the two men achieved glycemic control in 3-15 d without an additional medication[56]. However, no association between vaccination and disturbed glycemic control was proven.

Another study reported hyperglycemia between the 20th and 36th days after the first dose of the AstraZeneca vaccine[55]. Similar conditions have also been reported following mRNA vaccines, Comirnaty (Pfizer/BioNTech) and Spikevax (Moderna)[53]. This case report demonstrated remarkably high blood sugar and HbA1c levels after vaccination in a patient with previously reasonable blood glucose control. However, this patient probably had undiagnosed diabetes since his two parents had type 2 diabetes and the patient himself had a clinical picture of insulin resistance[53,57].

Another retrospective study examined 96 adults over the age of 18 with type 1 diabetes before and after their first COVID-19 vaccination[58]. Fifty-nine percent of them had a significant deviation in blood glucose levels, which were controlled within 7-10 d after vaccination. Again, the data show no difference in the effects between the AstraZeneca and Pfizer vaccines.

There could be many reasons for fluctuations in blood glucose. Regarding existing studies, no excipients and/or adjuvants to vaccines have been reported to cause hyperglycemia, so that the condition could be related to the antigens in the vaccine against COVID-19. A possible mechanism for its occurrence is stimulating the immune system, which leads to a stress response. However, it is milder than usually occurs with COVID-19 infection. Different changes in the glycolytic pathway occur in COVID-19 infection in response to stress and lead to increased glucose levels in cells[54,59]. Stress also increases hormones such as adrenaline, cortisol, and/or glucagon that cause metabolism changes[60]. In addition, they affect the immune system by reducing the activity and number of natural killer cells and lymphocytes, decreasing antibodies and reactivating latent viral infections[61].

More research and patient results need to be analyzed to provide a clear and definitive answer about this temporary instability of blood glucose levels after the COVID-19 vaccination. Understanding changes in the glycolytic pathway associated with COVID-19 and/or after vaccination could help find a new treatment for this disease.

Clinical data support a strong response of the neutralizing antibodies in patients with diabetes after COVID-19[36]. However, patients should be consulted and prepared for possible hyperglycemia after the COVID-19 vaccination[60].

There is still no data on the effects on glucose levels after the second COVID-19 vaccination or booster dose. These studies are underway. The question remains if the immunity to vaccination against COVID-19 in people with diabetes would change or decrease.

COVID-19 VACCINES: DATA ON EFFECTIVENESS IN DIABETES PATIENTS

Although there is a high incidence of diabetes among populations, during the COVID-19 vaccine studies, patients with diabetes are usually rolled out. Therefore, we rely on the data from real-life studies from vaccinations after the vaccine approval.

Soetedjo *et al*[62], in their systematic review, managed to cover eight studies with a total of 64468 patients and 5156 patients with diabetes[62]. The vaccines included were BNT162b2 vaccine

(Pfizer/BioNTech), CoronaVac (Sinovac Life Sciences), Covishield™ (ChAdOx1-nCOV), and Covaxin™ (BBV-152). The effectiveness studies showed lower seropositivity and antibody responses following vaccination in diabetes patients than in healthy controls. This was observed from 1-4 wk after full COVID-19 vaccination.

The studies on the immunogenicity of COVID-19 patients with diabetes are presented in Table 1[63-72].

We can assume from the data that the seroconversion rates in diabetes patients following COVID-19 vaccination is lower, including lower antibody titers. However, the underlying reasons for that are not fully understood. It was proposed that impaired adaptive immune response in diabetes patients contributes to altered vaccination response[37]. Additionally, patients with diabetes had some immune alterations such as reduced circulating CD4+ cells, lymphocyte proliferation, and antigen presentation [67]. Immunological alterations in patients with diabetes are shown in Figure 1.

As we stated above, hyperglycemia at immunization may reduce the immune response. As a result, having sufficient glycemic control during the post-vaccination interval increases immunological response because strict glycemic control may predispose to a favorable immune response to the SARS-CoV-2 vaccine[67]. The host's ability to respond to infections and the formation of long-term immunological memory, including correct responses to immunizations, are both influenced by the immune system's steady degradation. Among other things, the adaptive immune system can be compromised by poor proliferation in response to antigenic stimulation, impaired generation of CD4+ T follicular helper cells, and a reduced capacity to generate effector lymphokines. Additionally, it is well-known that hyperglycemia induced glycosylated receptors on the immune cells lead to impaired immune cell function[67]. Immunological features and alterations of diabetes are shown in Figure 1.

In line with this, the leading cause of reduced immune response and protection after vaccination in diabetes patients remains relative immune deficiency. Other factors, such as poorly controlled diabetes, may indirectly impact the vaccines' efficacy and effectiveness. Thus, we must pay attention to hyperglycemia, which can influence clinical COVID-19 results and vaccination efficacy. This leads us to assume that maintaining proper glycemic control after immunization increases immunological response. Also, we anticipate that strict glycemic management will support the favorable immunological response to the SARS-CoV-2 vaccination. Therefore, glycemic management should be the standard during pandemics, which strengthens the role of diabetologists in vaccination program effectiveness[67].

Additionally, different vaccines elicited comparable results, as shown in Table 1. This is also valid for the adverse effects demonstrated in the next section of the paper.

To sum up, the vaccines' overall effectiveness could also be evaluated by the re-infection rate among patients with diabetes who were immunized against SARS-CoV-2. Generally, patients with diabetes were among the population of people with a higher risk of re-infection, both after natural infection or vaccination[73]. However, no particular numbers or percentages were cited for the re-infection rate after COVID-19 vaccination in diabetes patients, although the risk ratio for hospitalization due to re-infection was declared at 1.6[74]. The re-infection was less likely to occur in naturally infected or vaccinated people than in naïve patients. Therefore, the disease course was expected to be less severe in vaccinated diabetes patients.

COVID-19 VACCINES: DATA ON ADVERSE EFFECTS IN DIABETES PATIENTS

Although the benefits of vaccination against COVID-19 in diabetic patients are undeniable, we will try to systematize the information gathered in the literature on the side effects of COVID-19 vaccines. A recent study analyzing the side effects of the two mRNA COVID-19 vaccines (BNT162b2 mRNA and mRNA-1273) among 1245 healthcare workers described general and organ-specific symptoms after the first and/or second dose of mRNA vaccines in the United States. The common endocrine symptoms were decreased appetite (5.73%), heat/cold intolerance (3.24%), increased thirst (1.12%), increased appetite (0.87%), and increased urine production (0.25%)[75]. Importantly, there are no reported symptoms associated with glucose metabolism; nevertheless, there is no information about diabetic participants in this study. Commonly reported symptoms were soreness, fatigue, myalgia, headache, chills, fever, joint pain, nausea, muscle spasm, sweating, dizziness, flushing, feeling of relief, brain fogging, anorexia, localized swelling, decreased sleep quality, itching, tingling, diarrhea, nasal stuffiness, and palpitations. Despite this extended list of symptoms, 79.7% of participants did not violate daily activities. In comparison, around 98.0% of them planned to have the second dose, and 92.9% had already received it[75].

An anaphylactic reaction is another reported side effect post-vaccination. The CDC reported in January 2021 that anaphylaxis might occur more frequently after the BNT162b2 mRNA vaccine than other vaccines[76]. According to this report, 11.1 per million was the estimated rate of anaphylaxis from 1893360 first doses of the Pfizer-BioNTech COVID-19 vaccine. The total reported adverse events after vaccination were 4393 (0.2%). Of them, only 175 cases were identified as potentially life-threatening allergic reactions, and 21 were reported as anaphylaxis. Most of the observed allergic reactions have

Table 1 Existing studies on effectiveness of coronavirus disease 2019 vaccines in patients with diabetes

Ref.	Type of study	Type and name of the vaccine	Participants	Efficacy/effectiveness	Adverse effects
Nomura *et al*[63]	Observational study	BNT162b2	12 from a total of 252, at a mean age of 43.9 yr	Lower antibody titers compared to non-diabetic subjects 3 mo post-vaccination	N/A
Lustig *et al* [64]	Longitudinal cohort study	BNT162b2	139 from a total of 2498, at a mean age of 47.7 yr; mostly healthcare workers	Substantial antibody response after 2 doses, but overall lower concentrations of IgG and IgA in diabetics compared to healthy adults	N/A
Van Praet *et al*[65]	Case-control study	BNT162b2	25 from a total of 75, at a mean age of 85 yr	Decreased cellular immune response only in individuals with diabetes or active malignancy in the studied population	N/A
Ali *et al*[66]	Cohort study	BNT162b2	81	The BNT162b2 vaccine induced robust IgG and neutralizing antibody responses in people with and without T2DM. On average, diabetics had 13.86 BAU/mL less IgG and 4.42% less neutralizing antibodies compared to non-diabetics	N/A
Marfella *et al*[67]	Prospective observational study	BNT162b2, mRNA-1273, ChAdOx1-S	251, of which 134 with optimal glycemic control and 117 with poor glycemic control	21 d after the second dose, neutralizing antibody titers and CD4 Th1 cytokine responses were weaker in individuals with HbA1c > 7% compared to those with HbA1c < 7% whose titers were indistinguishable from those of healthy subjects	N/A
Singh *et al* [68]	Cross-sectional study	ChAdOx1-nCOV (Covishield), BBV-152 (Covaxin)	52 from a total of 463 at a mean age of 44.8 yr	Amongst all studied comorbidities, people with T2DM had lower seropositivity rates compared to those without (84.6% *vs* 96.1%)	N/A
Sauré *et al* [69]	Surveillance study	CoronaVac, BNT162b2	4626 from a total of 59987 people from Chile's population	IgG seropositivity was significantly lower in diabetics receiving the CoronaVac vaccine compared to healthy subjects	N/A
Piccini *et al* [70]	Retrospective cohort study	mRNA-1273BNT162b2	39	In adolescents and young adults with T1DM, vaccination with either product was safe and did not influence glycemic control	No serious adverse events were reported
Watanabe *et al*[71]	Observational study	Pfizer/BioNTech BNT162b2 vaccine	2 from a total of 66 at a mean age of 29 yr; mostly healthcare workers	Undetectable titers of anti-SARS-CoV-2 antibodies	N/A
Karamese and Tutuncu[72]	Cross-sectional study	CoronaVac	49 from a total of 186 people, at a mean age of 70.4 yr	Significantly lower levels of anti-SARS-CoV-2 antibodies in diabetes patients than in the controls	N/A

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IgG: Immunoglobulin G; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; N/A: Not applicable; BAU: Binding antibody units.

manifested within the first 30 min of vaccination. Anaphylaxis usually occurs in individuals with a history of allergies or a previous anaphylactic episode. Again, there is no information related to glucose disturbances or predisposition for allergic reactions among diabetes patients[76].

Another CDC report on the side effects of the two mRNA vaccines showed that the most frequently reported symptoms after vaccination were headache, fatigue, and dizziness. The rate of anaphylaxis was defined as rare (4.5 reported cases per million doses administered). There were no data on side effects associated with glucose metabolism and no evidence that vaccine symptoms were more pronounced in diabetics[77].

Increased risk of myocarditis and pericarditis has been reported after mRNA COVID-19 vaccination (Pfizer-BioNTech and Moderna)[78-82] and rarely after adenovirus vector-related vaccine[83,84]. Detailed analyses of these cases showed that myocarditis and pericarditis were more often in adolescents and young adult males. In addition, they were associated with multiple comorbidities, including obesity and hyperlipidemia[85].

An Italian study reported that the most frequent adverse events observed post-vaccination were vagal response (30%), anxiety reaction (24%), and dizziness (21%) among a total of 314671 vaccinated subjects. These side effects were predominantly observed in women and people with comorbidities; however, it is unclear whether diabetes was included[86].

Another study analyzed the adverse effects among 447346 reports 2 wk after vaccination with one of following three COVID-19 vaccines: 19462 Ad26.COV2.S (Janssen COVID-19 vaccine), 120580 mRNA-1273 (Moderna COVID-19 vaccine), and 100752 BNT162b2 (Pfizer-BioNTech COVID-19 vaccine). Headache, joint-related symptoms, muscle pain, musculoskeletal and connective tissue pain, nausea or

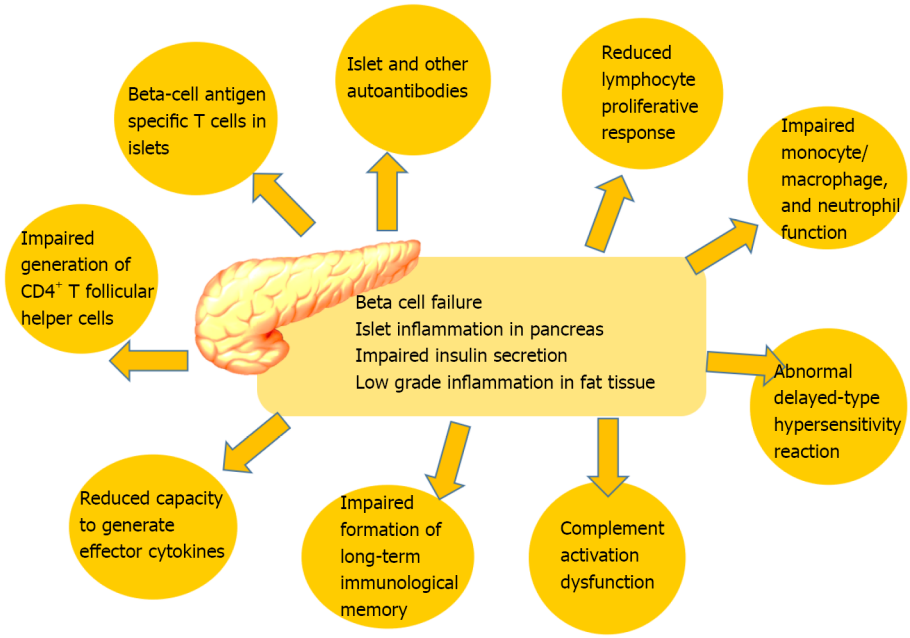

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Figure 1 Immunological alterations in diabetes patients.

vomiting, dermal and epidermal conditions, and febrile disorders were common post-vaccination complaints. They were associated with delayed recovery in people with underlying diseases, including diabetes[36].

Theoretically, vaccination could be followed by mild to moderate elevation of blood glucose levels [87]. However, a few studies have already reported worsened glucose control after the COVID-19 vaccination[53,56]. However, a recent study showed that COVID-19 vaccination was not associated with impairment in glucose management. The study analyzed the short-term effects of COVID-19 immunization in patients with type 1 and type 2 diabetes who were vaccinated with one of the following three COVID-19 vaccines: BNT162b2, mRNA-1273, and AZD1222 (Oxford-AstraZeneca). The study collected and analyzed 49200 continuous glucose monitoring data from 74 participants in the study and showed that there were no changes in time spent in the target glycemic range (70−180 mg/dL) on the days of follow-up (2 d before and 3 d after vaccination). However, patients with type 1 diabetes and more pronounced post-vaccine side effects spent more time above the target range (> 180 mg/dL); no such observations were found in patients with type 2 diabetes. Additionally, the study reported no need for adjustment in the insulin bolus dose and changes in carbohydrate intake around the vaccination in patients with both diabetes types[88].

To sum up, the most common systemic side effects are headache, chills, fever, flu-like symptoms, nausea, and fatigue. The local effects are pain, redness, and swelling at the injection site on the arm. Patients with diabetes are not more prone to have pronounced side effects of COVID-19 vaccination than healthy people. However, most of them are mild and disappear a few days after vaccine administration. Although it is possible to have increased blood sugar levels after vaccination, it is rather not associated with a significant impact on glycemic control. Therefore, it does not require any changes in diabetic therapy. However, we must remember that COVID-19 may exert deteriorating effects in patients with autoimmune diseases[89], including type 1 diabetes. On the other hand, therapies for type 2 diabetes that target cytokines can also change the course of infection[90].

VACCINE HESITANCY IN DIABETES PATIENTS

Diabetes patients were not excluded from the COVID-19 vaccine trials due to the higher prevalence of the disease amongst the populations. Thus, we obtained much more data on the evidence for the long-term safety and efficacy of the COVID-19 vaccine in patients with diabetes, in contrast to other autoimmune diseases where many gaps in the knowledge still exist. However, despite the considerable information, physicians and patients still fear exacerbation of the disease and the adverse effects of vaccination, which increases the hesitancy to vaccination.

Even though COVID-19 vaccination has emerged as the sole practical approach to improving clinical outcomes, vaccine hesitancy remains a barrier to obtaining significant levels of vaccine coverage. This poses a particular concern to individuals suffering from autoimmune illnesses, who are already at a

higher risk of hospitalization and poor clinical outcomes due to COVID-19 infection. While long-term safety and effectiveness data for COVID-19 immunization in individuals with autoimmune illnesses are lacking, existing research clearly shows that the advantages of vaccination exceed the risks of side effects and disease flare-ups.

The COVAD study group demonstrated some causes for vaccination hesitancy, which was reported in around half of the patients with autoimmune illnesses in the study's pilot findings[91]. Of all the respondents who did not receive any dose of the COVID-19 vaccine, 16.94% reported not getting the vaccine due to long-term safety concerns or other fears, such as disease exacerbation and delayed adverse effects, and 27.45% stated that they plan to wait until more data are available on the safety of the vaccine before vaccination. Other reasons given by the respondents for not vaccinating are the lack of the vaccine in some parts of the world (32.00%), planning for vaccination at a later date (11.67%), and postponing vaccination due to recent COVID-19 infection (7.30%). Some patients also reported not getting the vaccine because they had been advised to do so by their doctor (5.40%)[91].

However, there are no medical recommendations against vaccination. Only 35% of those vaccinated had mild side effects (fever/headache/myalgia). Furthermore, patients with autoimmune diseases had fewer side effects than healthy controls. Recent international studies show a negligible risk of severe side effects or disease exacerbation after vaccination[91].

Wang *et al*[91] showed that more than half of 483 Chinese diabetes patients experienced vaccine hesitancy (58.2%). Of them, 41.8% were unwilling to get the COVID-19 vaccine. Although patients were aware of the severity of COVID-19 in diabetes patients, they were concerned about vaccine safety. Interestingly, the vaccination status of their relatives did not influence the patients' decisions, but disagreement with their physician on the ability of the vaccine to reduce the severity of COVID-19 correlated with vaccine hesitancy[92].

The five factors associated with vaccine hesitancy in diabetes patients are the false belief that diabetes is not a high risk factor for severe COVID-19 and a lack of confidence in vaccine efficacy to prevent infection. However, diabetes patients were convinced that diabetes worsens COVID-19 prognosis and that vaccination may reduce the transmission risk. The third factor associated with vaccine hesitancy was the fear of adverse effects, and the fourth and fifth were the dependence on the opinion of others, including vaccines to be administered to a large group of people, and the influence of social media on them[92].

Similarly, Aldossari *et al*[92] showed that 34.7% of Saudi diabetes patients in the survey were willing to be vaccinated, and 79.0% supported COVID-19 vaccination. However, they showed signs of fear and uncertainty[92]. Therefore, the key to a successful vaccination campaign for these patients remains the accurate information provided and the fight against misinformation. Some factors related to vaccine hesitancy were relatively quick development, beliefs that the trials were insufficient, fears and uncertainty of components, and especially the mRNA behavior after vaccination. In addition, an enormous impact is the anti-vaccination movements in social and traditional media. Furthermore, social media misinformation has led to increased anxiety and vaccine hesitancy.

CONCLUSION

Since diabetes mellitus is a significant contributor to COVID-19 mortality, patients with disturbed glucose metabolism should be protected from SARS-CoV-2 infection. Few studies have established data on the effectiveness and safety of the COVID-19 vaccine in patients with diabetes. Despite the significant reduction in the immune response to vaccination in diabetes individuals, they should be prioritized for complete vaccination and booster dose delivery. Also, glycemic management in achieving sufficient immunity against SARS-CoV-2 is mandatory. Data also showed that COVID-19 vaccines presented an excellent safety profile with adverse effects following vaccination similar to the healthy population and no increase in the incidence of adverse events in the diabetic group. Finally, vaccine hesitancy among diabetes patients could be overcome with proper information and patient care.

FOOTNOTES

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REFERENCES

1 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]
2 **World Health Organization**. WHO Coronavirus (COVID-19) Dashboard. [cited 20 March 2022]. Available from: https://covid19.who.int/
3 **Ge XY**, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi ZL. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 2013; **503**: 535-538 [PMID: 24172901 DOI: 10.1038/nature12711]
4 **Haagmans BL**, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, Godeke GJ, Jonges M, Farag E, Diab A, Ghobashy H, Alhajri F, Al-Thani M, Al-Marri SA, Al Romaihi HE, Al Khal A, Bermingham A, Osterhaus AD, AlHajri MM, Koopmans MP. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infect Dis* 2014; **14**: 140-145 [PMID: 24355866 DOI: 10.1016/S1473-3099(13)70690-X]
5 **Walls AC**, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 2020; **181**: 281-292.e6 [PMID: 32155444 DOI: 10.1016/j.cell.2020.02.058]
6 **Perez-Saez J**, Lauer SA, Kaiser L, Regard S, Delaporte E, Guessous I, Stringhini S, Azman AS; Serocov-POP Study Group. Serology-informed estimates of SARS-CoV-2 infection fatality risk in Geneva, Switzerland. *Lancet Infect Dis* 2021; **21**: e69-e70 [PMID: 32679085 DOI: 10.1016/S1473-3099(20)30584-3]
7 **Verity R**, Okell LC, Dorigatti I, Winskill P, Whittaker C, Imai N, Cuomo-Dannenburg G, Thompson H, Walker PGT, Fu H, Dighe A, Griffin JT, Baguelin M, Bhatia S, Boonyasiri A, Cori A, Cucunubá Z, FitzJohn R, Gaythorpe K, Green W, Hamlet A, Hinsley W, Laydon D, Nedjati-Gilani G, Riley S, van Elsland S, Volz E, Wang H, Wang Y, Xi X, Donnelly CA, Ghani AC, Ferguson NM. Estimates of the severity of coronavirus disease 2019: a model-based analysis. *Lancet Infect Dis* 2020; **20**: 669-677 [PMID: 32240634 DOI: 10.1016/S1473-3099(20)30243-7]
8 **Henning RJ**. Type-2 diabetes mellitus and cardiovascular disease. *Future Cardiol* 2018; **14**: 491-509 [PMID: 30409037 DOI: 10.2217/fca-2018-0045]
9 **Donath MY**, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* 2011; **11**: 98-107 [PMID: 21233852 DOI: 10.1038/nri2925]
10 **Beckman JA**, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 2002; **287**: 2570-2581 [PMID: 12020339 DOI: 10.1001/jama.287.19.2570]
11 **Bahiru E**, Hsiao R, Phillipson D, Watson KE. Mechanisms and Treatment of Dyslipidemia in Diabetes. *Curr Cardiol Rep* 2021; **23**: 26 [PMID: 33655372 DOI: 10.1007/s11886-021-01455-w]
12 **Eckel RH**, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005; **365**: 1415-1428 [PMID: 15836891 DOI: 10.1016/S0140-6736(05)66378-7]
13 **Sun H**, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022; **183**: 109119 [PMID: 34879977 DOI: 10.1016/j.diabres.2021.109119]
14 **Vasbinder A**, Anderson E, Shadid H, Berlin H, Pan M, Azam TU, Khaleel I, Padalia K, Meloche C, O'Hayer P, Michaud E, Catalan T, Feroze R, Blakely P, Launius C, Huang Y, Zhao L, Ang L, Mikhael M, Mizokami-Stout K, Pennathur S, Kretzler M, Loosen SH, Chalkias A, Tacke F, Giamarellos-Bourboulis EJ, Reiser J, Eugen-Olsen J, Feldman EL, Pop-Busui R, Hayek SS; ISIC Study Group:, Hayek SS, Blakely P, Berlin H, Azam TU, Shadid H, Pan M, O'Hayer P, Meloche C, Feroze R, Padalia KJ, Anderson E, Perry D, Bitar A, Kaakati R, Huang Y, Zhao L, Reiser J, Samelko B, Hlepas A, Patel PP, Wang X, Eugen-Olsen J, Altintas I, Stauning M, Baltzer Houlind M, Lindstrøm MB, Gamst-Jensen H, Hartmann LJ, Nehlin JO, Kallemose T, Parvaiz I, Rasmussen C, Andersen O, Tingleff J, Giamarellos-Bourboulis EJ, Adami ME, Solomonidi N, Tsilika M, Saridaki M, Lekakis V, Loosen SH, Luedde T, Keitel V, Chalkias A, Arnaoutoglou E, Pantazopoulos I, Laou E, Kolonia K, Skoulakis A, Tacke F, Tober-Lau P, Mohr R, Kurth F, Sander LE, Jochum C. Inflammation, Hyperglycemia, and Adverse Outcomes in Individuals With Diabetes Mellitus Hospitalized for COVID-19. *Diabetes Care* 2022; **45**: 692-700 [PMID: 35045184 DOI: 10.2337/dc21-2102]
15 **Stokes EK**, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, Tie Y, Fullerton KE. Coronavirus Disease 2019 Case Surveillance - United States, January 22-May 30, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 759-765 [PMID: 32555134 DOI: 10.15585/mmwr.mm6924e2]
16 **CDC COVID-19 Vaccine Breakthrough Case Investigations Team**. COVID-19 Vaccine Breakthrough Infections

Reported to CDC - United States, January 1-April 30, 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70**: 792-793 [PMID: 34043615 DOI: 10.15585/mmwr.mm7021e3]

17 **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]

18 **Carey IM**, Critchley JA, DeWilde S, Harris T, Hosking FJ, Cook DG. Risk of Infection in Type 1 and Type 2 Diabetes Compared With the General Population: A Matched Cohort Study. *Diabetes Care* 2018; **41**: 513-521 [PMID: 29330152 DOI: 10.2337/dc17-2131]

19 **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]

20 **Holman N**, Knighton P, Kar P, O'Keefe J, Curley M, Weaver A, Barron E, Bakhai C, Khunti K, Wareham NJ, Sattar N, Young B, Valabhji J. Risk factors for COVID-19-related mortality in people with type 1 and type 2 diabetes in England: a population-based cohort study. *Lancet Diabetes Endocrinol* 2020; **8**: 823-833 [PMID: 32798471 DOI: 10.1016/S2213-8587(20)30271-0]

21 **Simonnet A**, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, Labreuche J, Mathieu D, Pattou F, Jourdain M; LICORN and the Lille COVID-19 and Obesity study group. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. *Obesity (Silver Spring)* 2020; **28**: 1195-1199 [PMID: 32271993 DOI: 10.1002/oby.22831]

22 **Green WD**, Beck MA. Obesity Impairs the Adaptive Immune Response to Influenza Virus. *Ann Am Thorac Soc* 2017; **14**: S406-S409 [PMID: 29161078 DOI: 10.1513/AnnalsATS.201706-447AW]

23 **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]

24 **Lim S**, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat Rev Endocrinol* 2021; **17**: 11-30 [PMID: 33188364 DOI: 10.1038/s41574-020-00435-4]

25 **Flue vaccination and Diabetes**. NHS. [cited 20 March 2022]. Available from: https://www.england.nhs.uk/south/wp-content/uploads/sites/6/2019/10/Diabetes-and-Flu-Vaccine-Importance.pdf

26 **CDC**. Influenza (Flu) Diabetes. [cited 20 March 2022]. Available from: https://www.cdc.gov/flu/highrisk/diabetes.htm#anchor_1537288125609

27 **Seo YB**, Baek JH, Lee J, Song JY, Lee JS, Cheong HJ, Kim WJ. Long-Term Immunogenicity and Safety of a Conventional Influenza Vaccine in Patients with Type 2 Diabetes. *Clin Vaccine Immunol* 2015; **22**: 1160-1165 [PMID: 26376926 DOI: 10.1128/CVI.00288-15]

28 **CDC**. Pneumococcal vaccine–Diabetes. [cited 20 March 2022]. Available from: https://www.cdc.gov/pneumococcal/vaccination.html

29 **Huijts SM**, van Werkhoven CH, Bolkenbaas M, Grobbee DE, Bonten MJM. Post-hoc analysis of a randomized controlled trial: Diabetes mellitus modifies the efficacy of the 13-valent pneumococcal conjugate vaccine in elderly. *Vaccine* 2017; **35**: 4444-4449 [PMID: 28410813 DOI: 10.1016/j.vaccine.2017.01.071]

30 **Perniciaro S**, van der Linden M. Pneumococcal vaccine uptake and vaccine effectiveness in older adults with invasive pneumococcal disease in Germany: A retrospective cohort study. *Lancet Reg Health Eur* 2021; **7**: 100126 [PMID: 34557837 DOI: 10.1016/j.lanepe.2021.100126]

31 **Chuanchaiyakul N**, Thongtang N, Rattanaumpawan P. Cumulative incidence of and risk factors for herpes zoster among patients with diabetes mellitus: Results from a 10-year nested case-control study. *J Diabetes Complications* 2022; **36**: 108168 [PMID: 35370058 DOI: 10.1016/j.jdiacomp.2022.108168]

32 **Thomas SL**, Hall AJ. What does epidemiology tell us about risk factors for herpes zoster? *Lancet Infect Dis* 2004; **4**: 26-33 [PMID: 14720565 DOI: 10.1016/s1473-3099(03)00857-0]

33 **Salzberger B**, Nitschmann S. [Herpes zoster vaccination efficacy in adults 70 years of age or older : ZOE-70 study]. *Internist (Berl)* 2017; **58**: 522-524 [PMID: 28357465 DOI: 10.1007/s00108-017-0222-3]

34 **Willer DO**, Oostvogels L, Cunningham AL, Gervais P, Gorfinkel I, Hyung Kim J, Talarico C, Wascotte V, Zahaf T, Colindres R, Schuind A; ZOE 50/70 study groups. Efficacy of the adjuvanted recombinant zoster vaccine (RZV) by sex, geographic region, and geographic ancestry/ethnicity: A post-hoc analysis of the ZOE-50 and ZOE-70 randomized trials. *Vaccine* 2019; **37**: 6262-6267 [PMID: 31537443 DOI: 10.1016/j.vaccine.2019.09.028]

35 **Godeaux O**, Kovac M, Shu D, Grupping K, Campora L, Douha M, Heineman TC, Lal H. Immunogenicity and safety of an adjuvanted herpes zoster subunit candidate vaccine in adults ≥ 50 years of age with a prior history of herpes zoster: A phase III, non-randomized, open-label clinical trial. *Hum Vaccin Immunother* 2017; **13**: 1051-1058 [PMID: 28068212 DOI: 10.1080/21645515.2016.1265715]

36 **Pal R**, Bhadada SK, Misra A. COVID-19 vaccination in patients with diabetes mellitus: Current concepts, uncertainties and challenges. *Diabetes Metab Syndr* 2021; **15**: 505-508 [PMID: 33662837 DOI: 10.1016/j.dsx.2021.02.026]

37 **Schillie SF**, Spradling PR, Murphy TV. Immune response of hepatitis B vaccine among persons with diabetes: a systematic review of the literature. *Diabetes Care* 2012; **35**: 2690-2697 [PMID: 23173138 DOI: 10.2337/dc12-0312]

38 **Theofilopoulos AN**, Kono DH, Baccala R. The multiple pathways to autoimmunity. *Nat Immunol* 2017; **18**: 716-724 [PMID: 28632714 DOI: 10.1038/ni.3731]

39 **Galeotti C**, Bayry J. Autoimmune and inflammatory diseases following COVID-19. *Nat Rev Rheumatol* 2020; **16**: 413-414 [PMID: 32499548 DOI: 10.1038/s41584-020-0448-7]

40 **Misra DP**, Agarwal V, Gasparyan AY, Zimba O. Rheumatologists' perspective on coronavirus disease 19 (COVID-19) and

potential therapeutic targets. *Clin Rheumatol* 2020; **39**: 2055-2062 [PMID: 32277367 DOI: 10.1007/s10067-020-05073-9]

41 **Velikova T**, Georgiev T. SARS-CoV-2 vaccines and autoimmune diseases amidst the COVID-19 crisis. *Rheumatol Int* 2021; **41**: 509-518 [PMID: 33515320 DOI: 10.1007/s00296-021-04792-9]

42 **Ahmed S**, Gasparyan AY, Zimba O. Comorbidities in rheumatic diseases need special consideration during the COVID-19 pandemic. *Rheumatol Int* 2021; **41**: 243-256 [PMID: 33388969 DOI: 10.1007/s00296-020-04764-5]

43 **Sen P**, Gupta L, Lilleker JB, Aggarwal V, Kardes S, Milchert M, Gheita T, Salim B, Velikova T, Gracia-Ramos AE, Parodis I, O'Callaghan AS, Nikiphorou E, Tan AL, Cavagna L, Saavedra MA, Shinjo SK, Ziade N, Knitza J, Kuwana M, Cagnotto G, Nune A, Distler O, Chinoy H, Aggarwal R; COVAD Study Group. COVID-19 vaccination in autoimmune disease (COVAD) survey protocol. *Rheumatol Int* 2022; **42**: 23-29 [PMID: 34779868 DOI: 10.1007/s00296-021-05046-4]

44 **Vadalà M**, Poddighe D, Laurino C, Palmieri B. Vaccination and autoimmune diseases: is prevention of adverse health effects on the horizon? *EPMA J* 2017; **8**: 295-311 [PMID: 29021840 DOI: 10.1007/s13167-017-0101-y]

45 **Salemi S**, D'Amelio R. Could autoimmunity be induced by vaccination? *Int Rev Immunol* 2010; **29**: 247-269 [PMID: 20521925 DOI: 10.3109/08830181003746304]

46 **Johns Hopkins**. Johns Hopkins Coronavirus research center. [cited 20 March 2022]. Available from: https://coronavirus.jhu.edu/map.html

47 **De Martino M**, Chiappini E, Galli L. Vaccines and autoimmunity. *Int J Immunopathol Pharmacol* 2013; **26**: 283-290 [PMID: 23755743 DOI: 10.1177/039463201302600201]

48 **Sakowicz-Burkiewicz M**, Kocbuch K, Grden M, Maciejewska I, Szutowicz A, Pawelczyk T. High glucose concentration impairs ATP outflow and immunoglobulin production by human peripheral B lymphocytes: involvement of P2X7 receptor. *Immunobiology* 2013; **218**: 591-601 [PMID: 22883563 DOI: 10.1016/j.imbio.2012.07.010]

49 **Huang I**, Lim MA, Pranata R. Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia - A systematic review, meta-analysis, and meta-regression. *Diabetes Metab Syndr* 2020; **14**: 395-403 [PMID: 32334395 DOI: 10.1016/j.dsx.2020.04.018]

50 **Gregory JM**, Slaughter JC, Duffus SH, Smith TJ, LeStourgeon LM, Jaser SS, McCoy AB, Luther JM, Giovannetti ER, Boeder S, Pettus JH, Moore DJ. COVID-19 Severity Is Tripled in the Diabetes Community: A Prospective Analysis of the Pandemic's Impact in Type 1 and Type 2 Diabetes. *Diabetes Care* 2021; **44**: 526-532 [PMID: 33268335 DOI: 10.2337/dc20-2260]

51 **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]

52 **Samuel SM**, Varghese E, Triggle CR, Büsselberg D. COVID-19 Vaccines and Hyperglycemia-Is There a Need for Postvaccination Surveillance? *Vaccines (Basel)* 2022; **10** [PMID: 35335086 DOI: 10.3390/vaccines10030454]

53 **Abu-Rumaileh MA**, Gharaibeh AM, Gharaibeh NE. COVID-19 Vaccine and Hyperosmolar Hyperglycemic State. *Cureus* 2021; **13**: e14125 [PMID: 33927933 DOI: 10.7759/cureus.14125]

54 **Santos AF**, Póvoa P, Paixão P, Mendonça A, Taborda-Barata L. Changes in Glycolytic Pathway in SARS-COV 2 Infection and Their Importance in Understanding the Severity of COVID-19. *Front Chem* 2021; **9**: 685196 [PMID: 34568275 DOI: 10.3389/fchem.2021.685196]

55 **Edwards AE**, Vathenen R, Henson SM, Finer S, Gunganah K. Acute hyperglycaemic crisis after vaccination against COVID-19: A case series. *Diabet Med* 2021; **38**: e14631 [PMID: 34185927 DOI: 10.1111/dme.14631]

56 **Mishra A**, Ghosh A, Dutta K, Tyagi K, Misra A. Exacerbation of hyperglycemia in patients with type 2 diabetes after vaccination for COVID19: Report of three cases. *Diabetes Metab Syndr* 2021; **15**: 102151 [PMID: 34186339 DOI: 10.1016/j.dsx.2021.05.024]

57 **Lee HJ**, Sajan A, Tomer Y. Hyperglycemic Emergencies Associated With COVID-19 Vaccination: A Case Series and Discussion. *J Endocr Soc* 2021; **5**: bvab141 [PMID: 34604689 DOI: 10.1210/jendso/bvab141]

58 **Heald AH**, Stedman M, Horne L, Rea R, Whyte M, Gibson JM, Livingston M, Anderson SG, Ollier W. Analysis of Continuous Blood Glucose Data in People with Type 1 Diabetes (T1DM) After COVID-19 Vaccination Indicates a Possible Link Between the Immune and the Metabolic Response. *J Diabetes Sci Technol* 2021; **15**: 1204-1205 [PMID: 34323111 DOI: 10.1177/19322968211026291]

59 **Saleh J**, Peyssonnaux C, Singh KK, Edeas M. Mitochondria and microbiota dysfunction in COVID-19 pathogenesis. *Mitochondrion* 2020; **54**: 1-7 [PMID: 32574708 DOI: 10.1016/j.mito.2020.06.008]

60 **Mifsud S**, Schembri EL, Gruppetta M. Stress-induced hyperglycaemia. *Br J Hosp Med (Lond)* 2018; **79**: 634-639 [PMID: 30418830 DOI: 10.12968/hmed.2018.79.11.634]

61 **Webster Marketon JI**, Glaser R. Stress hormones and immune function. *Cell Immunol* 2008; **252**: 16-26 [PMID: 18279846 DOI: 10.1016/j.cellimm.2007.09.006]

62 **Soetedjo NNM**, Iryaningrum MR, Lawrensia S, Permana H. Antibody response following SARS-CoV-2 vaccination among patients with type 2 diabetes mellitus: A systematic review. *Diabetes Metab Syndr* 2022; **16**: 102406 [PMID: 35104750 DOI: 10.1016/j.dsx.2022.102406]

63 **Nomura Y**, Sawahata M, Nakamura Y, Kurihara M, Koike R, Katsube O, Hagiwara K, Niho S, Masuda N, Tanaka T, Sugiyama K. Age and Smoking Predict Antibody Titres at 3 Months after the Second Dose of the BNT162b2 COVID-19 Vaccine. *Vaccines (Basel)* 2021; **9** [PMID: 34579279 DOI: 10.3390/vaccines9091042]

64 **Lustig Y**, Sapir E, Regev-Yochay G, Cohen C, Fluss R, Olmer L, Indenbaum V, Mandelboim M, Doolman R, Amit S, Mendelson E, Ziv A, Huppert A, Rubin C, Freedman L, Kreiss Y. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. *Lancet Respir Med* 2021; **9**: 999-1009 [PMID: 34224675 DOI: 10.1016/S2213-2600(21)00220-4]

65 **Van Praet JT**, Vandecasteele S, De Roo A, Vynck M, De Vriese AS, Reynders M. Dynamics of the cellular and humoral immune response after BNT162b2 mRNA Covid-19 vaccination in Covid-19 naive nursing home residents. *J Infect Dis* 2021 [PMID: 34514509 DOI: 10.1093/infdis/jiab458]

66 **Ali H**, Alterki A, Sindhu S, Alahmad B, Hammad M, Al-Sabah S, Alghounaim M, Jamal MH, Aldei A, Mairza MJ, Husain

M, Deverajan S, Ahmad R, Cherian P, Alkhairi I, Alkandari A, Abubaker J, Abu-Farha M, Al-Mulla F. Robust Antibody Levels in Both Diabetic and Non-Diabetic Individuals After BNT162b2 mRNA COVID-19 Vaccination. *Front Immunol* 2021; **12**: 752233 [PMID: 34899701 DOI: 10.3389/fimmu.2021.752233]

67 **Marfella R**, D'Onofrio N, Sardu C, Scisciola L, Maggi P, Coppola N, Romano C, Messina V, Turriziani F, Siniscalchi M, Maniscalco M, Boccalatte M, Napolitano G, Salemme L, Marfella LV, Basile E, Montemurro MV, Papa C, Frascaria F, Papa A, Russo F, Tirino V, Papaccio G, Galdiero M, Sasso FC, Barbieri M, Rizzo MR, Balestrieri ML, Angelillo IF, Napoli C, Paolisso G. Does poor glycaemic control affect the immunogenicity of the COVID-19 vaccination in patients with type 2 diabetes: The CAVEAT study. *Diabetes Obes Metab* 2022; **24**: 160-165 [PMID: 34494705 DOI: 10.1111/dom.14547]

68 **Singh AK**, Phatak SR, Singh R, Bhattacharjee K, Singh NK, Gupta A, Sharma A. Antibody response after first and second-dose of ChAdOx1-nCOV (Covishield™®) and BBV-152 (Covaxin™®) among health care workers in India: The final results of cross-sectional coronavirus vaccine-induced antibody titre (COVAT) study. *Vaccine* 2021; **39**: 6492-6509 [PMID: 34600747 DOI: 10.1016/j.vaccine.2021.09.055]

69 **Sauré D**, O'Ryan M, Torres JP, Zuniga M, Santelices E, Basso LJ. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: a sentinel surveillance study. *Lancet Infect Dis* 2022; **22**: 56-63 [PMID: 34509185 DOI: 10.1016/S1473-3099(21)00479-5]

70 **Piccini B**, Pessina B, Pezzoli F, Casalini E, Toni S. COVID-19 vaccination in adolescents and young adults with type 1 diabetes: Glycemic control and side effects. *Pediatr Diabetes* 2022; **23**: 469-472 [PMID: 35150596 DOI: 10.1111/pedi.13326]

71 **Watanabe M**, Balena A, Tuccinardi D, Tozzi R, Risi R, Masi D, Caputi A, Rossetti R, Spoltore ME, Filippi V, Gangitano E, Manfrini S, Mariani S, Lubrano C, Lenzi A, Mastroianni C, Gnessi L. Central obesity, smoking habit, and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine. *Diabetes Metab Res Rev* 2022; **38**: e3465 [PMID: 33955644 DOI: 10.1002/dmrr.3465]

72 **Karamese M**, Tutuncu EE. The effectiveness of inactivated SARS-CoV-2 vaccine (CoronaVac) on antibody response in participants aged 65 years and older. *J Med Virol* 2022; **94**: 173-177 [PMID: 34427924 DOI: 10.1002/jmv.27289]

73 **Ren X**, Zhou J, Guo J, Hao C, Zheng M, Zhang R, Huang Q, Yao X, Li R, Jin Y. Reinfection in patients with COVID-19: a systematic review. *Glob Health Res Policy* 2022; **7**: 12 [PMID: 35488305 DOI: 10.1186/s41256-022-00245-3]

74 **Rahman S**, Rahman MM, Miah M, Begum MN, Sarmin M, Mahfuz M, Hossain ME, Rahman MZ, Chisti MJ, Ahmed T, Arifeen SE, Rahman M. COVID-19 reinfections among naturally infected and vaccinated individuals. *Sci Rep* 2022; **12**: 1438 [PMID: 35082344 DOI: 10.1038/s41598-022-05325-5]

75 **Kadali RAK**, Janagama R, Peruru S, Malayala SV. Side effects of BNT162b2 mRNA COVID-19 vaccine: A randomized, cross-sectional study with detailed self-reported symptoms from healthcare workers. *Int J Infect Dis* 2021; **106**: 376-381 [PMID: 33866000 DOI: 10.1016/j.ijid.2021.04.047]

76 **CDC COVID-19 Response Team**; Food and Drug Administration. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Pfizer-BioNTech COVID-19 Vaccine - United States, December 14-23, 2020. *MMWR Morb Mortal Wkly Rep* 2021; **70**: 46-51 [PMID: 33444297 DOI: 10.15585/mmwr.mm7002e1]

77 **Gee J**, Marquez P, Su J, Calvert GM, Liu R, Myers T, Nair N, Martin S, Clark T, Markowitz L, Lindsey N, Zhang B, Licata C, Jazwa A, Sotir M, Shimabukuro T. First Month of COVID-19 Vaccine Safety Monitoring - United States, December 14, 2020-January 13, 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70**: 283-288 [PMID: 33630816 DOI: 10.15585/mmwr.mm7008e3]

78 **Abu Mouch S**, Roguin A, Hellou E, Ishai A, Shoshan U, Mahamid L, Zoabi M, Aisman M, Goldschmid N, Berar Yanay N. Myocarditis following COVID-19 mRNA vaccination. *Vaccine* 2021; **39**: 3790-3793 [PMID: 34092429 DOI: 10.1016/j.vaccine.2021.05.087]

79 **Albert E**, Aurigemma G, Saucedo J, Gerson DS. Myocarditis following COVID-19 vaccination. *Radiol Case Rep* 2021; **16**: 2142-2145 [PMID: 34025885 DOI: 10.1016/j.radcr.2021.05.033]

80 **Bautista García J**, Peña Ortega P, Bonilla Fernández JA, Cárdenes León A, Ramírez Burgos L, Caballero Dorta E. Acute myocarditis after administration of the BNT162b2 vaccine against COVID-19. *Rev Esp Cardiol (Engl Ed)* 2021; **74**: 812-814 [PMID: 33994339 DOI: 10.1016/j.rec.2021.04.005]

81 **Ammirati E**, Cavalotti C, Milazzo A, Pedrotti P, Soriano F, Schroeder JW, Morici N, Giannattasio C, Frigerio M, Metra M, Camici PG, Oliva F. Temporal relation between second dose BNT162b2 mRNA Covid-19 vaccine and cardiac involvement in a patient with previous SARS-COV-2 infection. *Int J Cardiol Heart Vasc* 2021; **34**: 100774 [PMID: 33821210 DOI: 10.1016/j.ijcha.2021.100774]

82 **Marshall M**, Ferguson ID, Lewis P, Jaggi P, Gagliardo C, Collins JS, Shaughnessy R, Caron R, Fuss C, Corbin KJE, Emuren L, Faherty E, Hall EK, Di Pentima C, Oster ME, Paintsil E, Siddiqui S, Timchak DM, Guzman-Cottrill JA. Symptomatic Acute Myocarditis in 7 Adolescents After Pfizer-BioNTech COVID-19 Vaccination. *Pediatrics* 2021; **148** [PMID: 34088762 DOI: 10.1542/peds.2021-052478]

83 **D'Angelo T**, Cattafi A, Carerj ML, Booz C, Ascenti G, Cicero G, Blandino A, Mazziotti S. Myocarditis After SARS-CoV-2 Vaccination: A Vaccine-Induced Reaction? *Can J Cardiol* 2021; **37**: 1665-1667 [PMID: 34118375 DOI: 10.1016/j.cjca.2021.05.010]

84 **Nassar M**, Nso N, Gonzalez C, Lakhdar S, Alshamam M, Elshafey M, Abdalazeem Y, Nyein A, Punzalan B, Durrance RJ, Alfishawy M, Bakshi S, Rizzo V. COVID-19 vaccine-induced myocarditis: Case report with literature review. *Diabetes Metab Syndr* 2021; **15**: 102205 [PMID: 34293552 DOI: 10.1016/j.dsx.2021.102205]

85 **Gianfredi V**, Minerva M, Casu G, Capraro M, Chiecca G, Gaetti G, Mantecca Mazzocchi R, Musarò P, Berardinelli P, Basteri P, Bertini B, Ferri C, Odone A, Signorelli C, Alberti VF, Gastaldi G. Immediate adverse events following COVID-19 immunization. A cross-sectional study of 314,664 Italian subjects. *Acta Biomed* 2021; **92**: e2021487 [PMID: 34739452 DOI: 10.23750/abm.v92iS6.12365]

86 **Son CS**, Jin SH, Kang WS. Propensity-Score-Matched Evaluation of Adverse Events Affecting Recovery after COVID-19 Vaccination: On Adenovirus and mRNA Vaccines. *Vaccines (Basel)* 2022; **10** [PMID: 35214742 DOI: 10.3390/vaccines10020284]

87 **Aberer F**, Moser O, Aziz F, Sourij C, Ziko H, Lenz J, Abbas F, Obermayer AM, Kojzar H, Pferschy PN, Müller A,

Unteregger C, Leitner M, Banfic T, Eckstein ML, Wachsmuth N, Kaser S, Mader JK, Tripolt NJ, Sourij H. Impact of COVID-19 Vaccination on Glycemia in Individuals With Type 1 and Type 2 Diabetes: Substudy of the COVAC-DM Study. *Diabetes Care* 2022; **45**: e24-e26 [PMID: 34848490 DOI: 10.2337/dc21-1563]

88 **Georgiev T**, Angelov AK. Complexities of diagnosis and management of COVID-19 in autoimmune diseases: Potential benefits and detriments of immunosuppression. *World J Clin Cases* 2020; **8**: 3669-3678 [PMID: 32953843 DOI: 10.12998/wjcc.v8.i17.3669]

89 **Velikova TV**, Kabakchieva PP, Assyov YS, Georgiev TA. Targeting Inflammatory Cytokines to Improve Type 2 Diabetes Control. *Biomed Res Int* 2021; **2021**: 7297419 [PMID: 34557550 DOI: 10.1155/2021/7297419]

90 **Sen P**, Lilleker JB, Agarwal V, Kardes S, Milchert M, Gheita T, Salim B, Velikova T, Gracia-Ramos AE, Parodis I, O'Callaghan AS, Nikiphorou E, Tan AL, Cavagna L, Saavedra MA, Shinjo SK, Ziade N, Knitza J, Kuwana M, Cagnotto G, Nune A, Distler O, Chinoy H, Aggarwal R, Gupta L. COVAD Study Group. Vaccine Hesitancy in Patients with Autoimmune Diseases- Data from the COVID-19 Vaccination in Autoimmune Diseases (COVAD) Study'. *Indian J Rheumatol* 2022; **17**: 188-191 [DOI: 10.4103/injr.injr_221_21]

91 **Wang Y**, Duan L, Li M, Wang J, Yang J, Song C, Li J, Jia J, Xu J. COVID-19 Vaccine Hesitancy and Associated Factors among Diabetes Patients: A Cross-Sectional Survey in Changzhi, Shanxi, China. *Vaccines (Basel)* 2022; **10** [PMID: 35062790 DOI: 10.3390/vaccines10010129]

92 **Aldossari KK**, Alharbi MB, Alkahtani SM, Alrowaily TZ, Alshaikhi AM, Twair AA. COVID-19 vaccine hesitancy among patients with diabetes in Saudi Arabia. *Diabetes Metab Syndr* 2021; **15**: 102271 [PMID: 34500380 DOI: 10.1016/j.dsx.2021.102271]

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ORIGINAL ARTICLE

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Hyperglycemia and reduced adiposity of streptozotocin-induced diabetic mice are not alleviated by oral benzylamine supplementation

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Abstract

BACKGROUND

Benzylamine (Bza) oral administration delays the onset of hyperglycemia in insulin-resistant *db*$^{-/-}$ mice; a genetic model of obesity and type 2 diabetes.

AIM

To extend the antihyperglycemic properties of oral benzylamine to a model of insulin-deficient type 1 diabetes.

METHODS

Male Swiss mice were rendered diabetic by streptozotocin treatment (STZ) and divided in two groups: one received 0.5% Bza as drinking solution for 24 d (STZ Bza-drinking) while the other was drinking water *ad libitum*. Similar groups were constituted in age-matched, nondiabetic mice. Food intake, liquid intake, body weight gain and nonfasting blood glucose levels were followed during treatment. At the end of treatment, fasted glycemia, liver and white adipose tissue (WAT) mass were measured, while glucose uptake assays were performed in adipocytes.

RESULTS

STZ diabetic mice presented typical features of insulin-deficient diabetes: reduced body mass and increased blood glucose levels. These altered parameters were not normalized in the Bza-drinking group in spite of restored food and water intake.

Bza consumption could not reverse the severe fat depot atrophy of STZ diabetic mice. In the nondiabetic mice, no difference was found between control and Bza-drinking mice for any parameter. In isolated adipocytes, hexose uptake was partially activated by 0.1 mmol/L Bza in a manner that was obliterated *in vitro* by the amine oxidase inhibitor phenelzine and that remained unchanged after Bza supplementation. Oxidation of 0.1 mmol/L Bza in WAT was lower in STZ diabetic than in normoglycemic mice.

CONCLUSION

Bza supplementation could not normalize the altered glucose handling of STZ diabetic mice with severe WAT atrophy. Consequently, its antidiabetic potential in obese and diabetic rodents does not apply to lipoatrophic type 1 diabetic mice.

Key Words: Diabetes; Adipocytes; Amine oxidases; Insulin-like agents; Glucose transport; Polydipsia

Core Tip: In adipocytes, benzylamine (Bza) is oxidized by amine oxidases and stimulates glucose uptake. Bza oral administration alleviates insulin-resistant diabetes in obese and diabetic mice. It was investigated whether Bza was also antihyperglycemic in insulin-deficient type 1 diabetes. To this aim, a 0.5% Bza drinking solution was given to streptozotocin-induced diabetic mice. Oral Bza did not recover hyperglycemia and reduced adiposity of lipoatrophic and diabetic mice. A minimal level of adiposity was required to support benzylamine oxidation and to improve glucose utilization. Thus, the antidiabetic properties of Bza in obese and diabetic models, do not apply for diabetes with severe lipoatrophy.

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INTRODUCTION

A recent study indicates that orally given benzylamine (Bza) delays the onset of diabetes in obese and insulin-resistant $db^{-/-}$ mice[1]. Supplementation with 0.5% Bza (5 g/L) in the drinking water impaired the increase in blood glucose, water intake and urine emission that occurs after weaning in this mouse model of insulin-resistant type 2 diabetes. The proposed mechanism of action for ingested Bza, which is naturally present in vegetables and edible plants, relies on its oxidation by an amine oxidase, which is a copper-containing enzyme highly expressed in fat cells[2,3]: The semicarbazide-sensitive amine oxidase (SSAO)[4] also known as amine oxidase copper containing 3[5] and identical to vascular adhesion protein (VAP-1)[6]. More precisely, it is hydrogen peroxide, one of the products of amine oxidation, and known from decades to stimulate glucose uptake in fat cells[7], that supports the insulin-mimetic actions of Bza in adipocytes, either in rodents[8] or in humans[9]. The *in vitro* insulin-like actions of Bza encompass activation of glucose uptake[10], induction of adipogenesis[11] , stimulation of lipogenesis [12], and inhibition of lipolysis. They occur even in the absence of insulin[8]. It was therefore of interest to investigate whether an oral treatment with Bza is capable of alleviating the impaired glucose handling of insulin-deficient, type 1 diabetic states.

Type 1 diabetes is characterized by a deficiency in insulin resulting from endocrine pancreas injury. To treat this disease, it is necessary to permanently normalize the altered blood glucose homeostasis. Since insulin is the major regulator of blood glucose levels, many therapeutic beneficial approaches have consisted in providing this pancreatic hormone, *via* repeated injections, or even by more sophisticated administration modes using biotechnologies, islet transplants or cell therapies[13]. Whatever the mode of supply, insulin overdose has to be avoided to prevent the risk of fatal hypoglycemia and to limit the onset of insulin resistance. Of note, various pharmacological agents or naturally occurring molecules can act as insulin-like factors on the glucose utilization by peripheral tissues[14]. In this view, testing the putative antihyperglycemic effect of Bza in type 1 diabetic rodents remains a preclinical step that deserves descriptive studies.

Alongside its capacity to oxidize Bza[1], fat tissue is not quantitatively but qualitatively of paramount importance in the regulation of glucose disposal. Adipose tissue uses glucose for accumulating lipid stores, and it also acts as an endocrine organ secreting a variety of adipokines with hyperglycemic or hypoglycemic properties, even in the absence of exogenous insulin[15]. The lack of adipose tissue

(lipoatrophy), such as that obtained in several genetically modified mice, is accompanied with altered glucose homeostasis[16,17]. Similarly, diabetic type 1 models, such as streptozotocin (STZ) diabetic rodents, with destroyed endocrine pancreas, exhibit reduced fat stores[18,19]. In humans, successful treatment of type 1 diabetes is concomitant with both restoration of normal glucose levels and adipose tissue recovery[20].

More importantly, diabetic phenotypes of diverse animal models have been ameliorated when white adipose tissue (WAT) or brown adipose tissue (BAT) has been reintroduced in these models, irrespective of the method used. Nowadays, it is suggested that adipose tissue contributes to the correction of type 1 diabetes, since hyperglycemia was lowered in diabetic mice treated by conditioned media from adipose-derived stem cells[21], and since mitigation of diabetes was observed in STZ diabetic mice receiving BAT transplantation[19]. To date, the beneficial effects of ingested Bza on glucose and lipid handling have been studied in obese rodents only[1,22]. These studies have suggested that enhanced fat deposition contribute to the insulin-like effects observed *in vivo*. Again, these considerations reinforced our interest in investigating the effects of Bza in a lipoatrophic model of type 1 diabetes.

The capacity of Bza to activate glucose transport in rat or mouse adipocytes is potentiated by the presence of vanadium[10,23], a widely recognized insulin-like agent[24,25]. Accordingly, it has been already demonstrated that *in vivo* treatments with a combination of amine oxidase substrates and vanadium exert antidiabetic effects in diverse diabetic rodents, including the STZ diabetic rats[10,26]. However, we demonstrated in recent studies that Bza[9] or catecholamines[27] are capable of activating glucose transport in human adipocytes, even in the absence of vanadium, and that the synergism vanadate/amine is much more weak in human adipocytes than in the murine ones. All these observations prompted us to examine for the first time the influence of prolonged oral administration of Bza alone-without any added vanadate—in a model of type 1 diabetes, which is nonobese and insulin-deficient; the STZ-induced diabetic mouse.

We investigated whether Bza alone was able, *via* oral consumption, to improve glucose handling in insulin-deficient STZ mice. The following results do not confirm our assumption, although they suggest that Bza action on glucose disposal requires a minimal amount of adipocytes prone to increase their glucose consumption when oxidizing this SSAO substrate.

MATERIALS AND METHODS

Chemicals

Benzylamine hydrochloride, STZ, bovine insulin, phenelzine, collagenase A, and most of the other reagents were from Sigma–Aldrich–Merck (Saint Quentin Fallavier, France). [^{3}H]-2-Deoxyglucose (2-DG) was from Perkin Elmer (Boston, MA, USA). The glucometers and consumables for follow-up of fed blood glucose were provided by Pr. Valet P. (Univ Toulouse, France), and used as previously described [28].

Insulin-deficient type 1 diabetic mice

Male Swiss mice obtained from Charles River Laboratories (L'arbresle, France) were housed at constant temperature (20–22°C) and with a 12-h light–dark cycle. At the age of 2 mo, they received an intraperitoneal injection of STZ (40 mg/kg) diluted in citrate buffer (0.05 mmol/L, pH 4.5) for four consecutive days, as described previously[21]. A week later, mice receiving only citrate buffer (nondiabetic) and treated mice exhibiting blood glucose ≥ 300 mg/100 mL (STZ diabetic) were subdivided into four groups of eight males, with either free access to water (control) or a 0.5% Bza solution as drinking liquid (Bza-drinking) for 24 d. To measure plasma insulin levels at the beginning of treatment, blood samples were withdrawn from tail vein then centrifuged and analyzed using Ultrasensitive insulin-ELISA kit (Mercodia, Uppsala, Sweden). All the mice had free access to food and water and were treated in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments)[29]. During this period, nonfasting blood glucose levels were determined every 3 d at 12:00 h (equivalent in the used circadian rhythm to 4 h after lights turned on) using an Accu-Check glucometer (Roche Diagnostics) on a blood drop withdrawn from the tail vein. Mice were killed after overnight fasting at the end of treatment and organs were collected and weighed.

Adipocyte preparations

Adipocyte preparations were obtained by collagenase digestion of WAT immediately after removal from the epididymal, intra-abdominal and inguinal anatomical locations. WAT was cut into small pieces, digested at 37°C by collagenase under agitation in Krebs–Ringer buffered at pH 7.5 with 15 mmol/L sodium bicarbonate, 10 mmol/L HEPES, supplemented with 3.5% of bovine serum albumin, as previously described[1]. Preparations of buoyant adipocytes were isolated from the digested WAT by filtration through nylon stockings and two gentle buffer washes, as described previously[10]. In our digestion process, approximately 1 g WAT was necessary to obtain sufficient functional adipocytes for the subsequent hexose uptake assays. When total amount of dissected WAT exceeded 1 g, excess

samples were snap-frozen at -80°C. This occurred for each of the normoglycemic mice but not for the lipoatrophic STZ-treated mice. In this case, pools of two mice were used to freeze approximately 200 mg WAT.

Glucose transport assays

The nonmetabolizable analog [^{3}H]-2-DG was the only source of hexose for the cell preparations during glucose transport assays. It was added at a final concentration of 0.1 mmol/L after 45 min incubation of the fat cell suspension with the tested agents, as previously described[10]. Pyruvate (2 mmol/L) was also present in the medium throughout the experiments for energy supply. Radioactive 2-DG (100 µL; approximately 1300000 dpm/vial) was added to 400 µL fat cell suspension, and hexose uptake assays were stopped 10 min later with 100 µL 100 µmol/L cytochalasin B. Cell suspensions (200 µL) were immediately transferred to plastic centrifugation microtubes prefilled with dinonyl-phthalate (density 0.98 g/mL), then subjected to a 30 s spin. The upper part of the tubes, containing radiolabelled hexose internalized in intact fat cells floating above the silicon layer was counted in scintillation vials, as described previously[10]. The extracellular [^{3}H]-2-DG present in the upper part of the tubes was determined in tubes receiving cytochalasin B prior to 2-DG. It averaged 1%–5% of the radioactivity found in control uptake, and was subtracted from all assays, as described previously[9].

Determination of benzylamine oxidation

Amine oxidase activity was determined at 37°C using [^{14}C]-Bza as substrate, in homogenates of thawed WAT samples, as previously described[10]. Isotopic dilution of [^{14}C]-Bza (final concentration: 0.1 mmol/L) was incubated for 30 min in 200 µL 200 mmol/L phosphate buffer with approximately 50 µg proteins, then the radiolabeled oxidation products were immediately extracted in toluene/ethyl acetate and counted as previously specified[9]. Results were expressed as nmol of deamination products/mg protein/min.

Statistical analysis

Results are presented as means ± SEM of (n) observations. All the statistical analyses for comparisons between parameters used ANOVA followed by *post hoc* Dunnett's multiple comparisons test, analyzed with Prism 6 for Mac OS X (GraphPad Software). Relative EC_{50} values were calculated by nonlinear regression.

RESULTS

Bza supplementation normalizes increased food and water consumption of STZ-induced diabetic mice without restoring body weight gain

At the start of the experiment, the STZ-induced diabetic mice exhibited lower body weight when compared to age-matched normoglycemic mice (Figure 1). The body weight gain of the insulin-deficient mice was also limited during the treatment period and was not corrected by Bza supplementation. At the end of the experiment, the mean body weight of STZ mice remained lower than that of normoglycemic mice. Hence, Bza supplementation tended to limit body weight gain in both groups, but this trend did not reach significance (Figure 1A). No significant decrease in food consumption was found in the Bza-drinking normoglycemic mice. By contrast, the hyperphagic status of the STZ mice was alleviated by Bza supplementation (Figure 1B). A similar influence of Bza supplementation was found for water consumption. An almost normalization of the elevated daily water intake of STZ diabetic mice occurred in the group subjected to Bza drinking (Figure 1B).

Figure 1 also shows that the characteristic polydipsic feature that occurs in STZ-induced type 1 diabetes was of greater magnitude than the hyperphagy triggered by the noxious diabetogenic agent. The exaggerated liquid consumption of the diabetic group was increased by 5.7 times when compared to normoglycemic control while this increase only reached 1.7 times for food intake. The former defect was expected to traduce glycosuria[19,30], while the second likely corresponded to a lowered efficiency of the ingested carbohydrates that accompanies insulin deficiency[31].

In view of these alterations of food and water intake in STZ diabetic mice and their recovery after Bza drinking, the influence of Bza supplementation on blood glucose levels was examined in both fed and fasted conditions.

Influence of oral supplementation of Bza on blood glucose in nondiabetic and diabetic mice

Figure 2 shows the pattern of nonfasting glycemia during the treatment period for the four experimental groups. The unfasted blood glucose levels of the mice previously challenged with STZ were at least twice higher than those of the controls throughout the study (Figure 2A). Such strong hyperglycemia was mainly a consequence of the low circulating levels of insulin found at the start of treatment in the two groups of STZ diabetic mice (0.40 ± 0.04 and 0.38 ± 0.05 ng/mL) when compared to the nondiabetic mice (1.26 ± 0.14 and 1.35 ± 0.09 ng/mL, n = 8; P < 0.001). In the STZ diabetic mice, the blood glucose

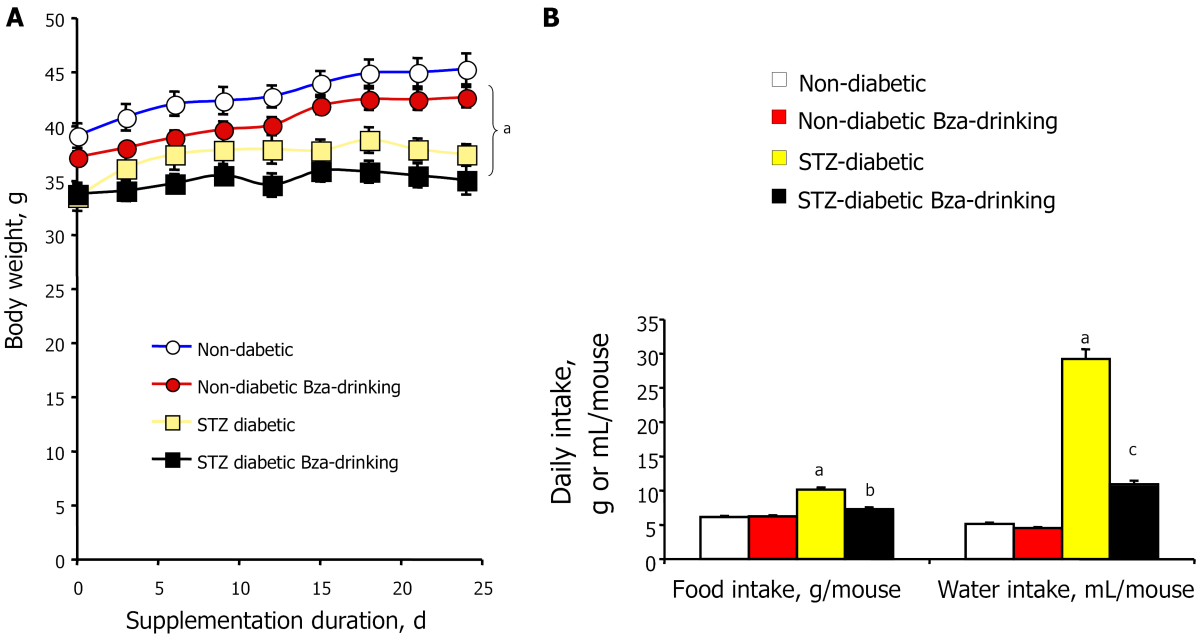

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Figure 1 Influence of Bza supplementation on body weight gain, food intake and water consumption in normoglycemic and STZ-induced diabetic mice. A: Body weight of diabetic (squares) and nondiabetic mice (circles) drinking water (open symbols) or 0.5% Bza (Bza-drinking, closed symbols). Mean ± SEM of n = 8 males in each group. Significant difference at: $^{a}P < 0.001$ between diabetic and nondiabetic mice, irrespective of the treatment; B: Average daily food intake and water intake. The mean daily consumption calculated throughout the treatment is expressed as g or mL/mouse, for each of the following groups: nondiabetic (white columns), nondiabetic Bza-drinking (red columns), STZ diabetic (yellow columns), STZ diabetic Bza-drinking (black columns). Each column is the mean ± SEM of at least 16 determinations. Different from nondiabetic rats at: $^{a}P < 0.01$. Different from respective control significant at: $^{b}P < 0.01$; $^{c}P < 0.001$. STZ: Streptozotocin; Bza: Benzylamine.

levels remained elevated in both Bza-drinking and water-drinking groups (Figure 2A). In the normoglycemic mice, the nonfasting blood glucose was superimposed in the control and Bza-drinking groups and remained below 200 mg/100 mL. Thus, blood glucose levels were not significantly influenced by repeated Bza consumption.

To avoid any alteration in body weight gain and in glucose handling, the mice were subjected to overnight fasting only once, at the end of experiment. Fasting blood levels were expectedly lower than nonfasting blood glucose (Figure 2B). Again, the fasting values were superimposable in Bza-drinking mice and their respective controls, while the fasting blood glucose of STZ diabetic mice was higher than that in nondiabetic groups (Figure 2B). Thus, Bza supplementation did not exhibit any hypoglycemic or antihyperglycemic action in this animal model of severe type 1 diabetes.

These findings contrasted with the capacity of Bza to delay the onset of diabetes in the genetically obese and diabetic *db*$^{-/-}$ mice[1]. Given the unexpected lack of efficiency of Bza consumption on glucose handling, it was poorly appropriate to delineate its putative mechanisms of action or to further examine other surrogate makers of diabetic state, as reported previously[1]. Instead, we verified whether the dose of Bza ingested was similar in the two diabetic models. Considering the daily liquid intake and the body mass of the STZ mice, it was calculated that these type 1 diabetic mice ingested 10850 ± 598 μmol/kg bw/d Bza throughout the treatment. This dose was similar to that used for Bza supplementation in young type 2 diabetic *db*$^{-/-}$ mice[1], which ranged between 9300 and 10 100 μmol/kg bw/d. However, another difference between type 2 (insulin-resistant) and type 1 (insulin-deficient) diabetic mouse models lies in the occurrence of excessive fat depots in the former and a clearly emaciated state in the latter. Therefore, attention was focused on WAT in the STZ mice and their controls.

Comparison of fat stores between normoglycemic and STZ-induced diabetic mice

Smaller mass of subcutaneous and visceral WAT was a typical feature of STZ-induced diabetic mice when compared to normoglycemic controls (Figure 3). In the STZ diabetic mice, the low mass of fat pads was not modified by Bza drinking, whatever their anatomical location. Similarly, the normal adiposity of the nondiabetic mice was not modified after oral Bza supplementation.

When the mass of the dissected fat depots was normalized as percentage of body weight, such adiposomatic index[22] was significantly lower in diabetic than in nondiabetic mice (1.2 ± 0.4% *vs* 3.7 ± 0.6%, $P < 0.001$). Again, Bza supplementation did not modify adiposomatic index: 1.3 ± 0.4% and 3.9 ± 0.4%, in Bza-drinking diabetic and nondiabetic groups, respectively.

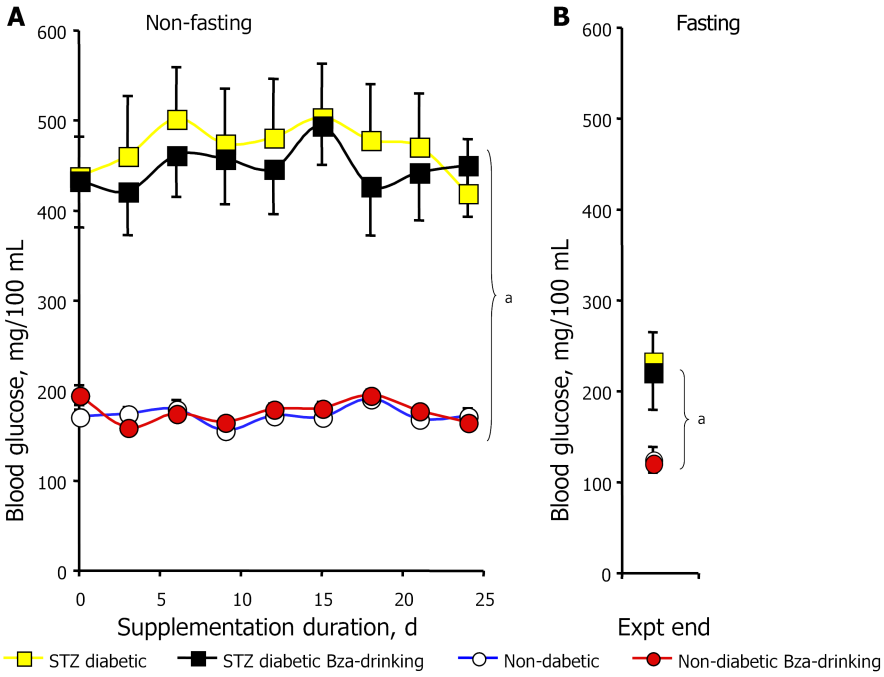

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Figure 2 Non-fasting and fasting blood glucose in normoglycemic and STZ-induced diabetic mice during Bza supplementation. A: Blood glucose measured every three days at 12:00 in diabetic (squares) and nondiabetic mice (circles) drinking water (open symbols) or Bza 0.5% (Bza-drinking, closed symbols) for 24 d; B: Overnight fasted blood glucose levels at the end of experiment for the same groups of mice. Mean ± SEM of n = 8 males in each group. Different from nondiabetic mice at: $^{a}P < 0.001$. No significant difference was found between Bza drinking and respective controls. STZ: Streptozotocin; Bza: Benzylamine; Expt end: The end of experiment.

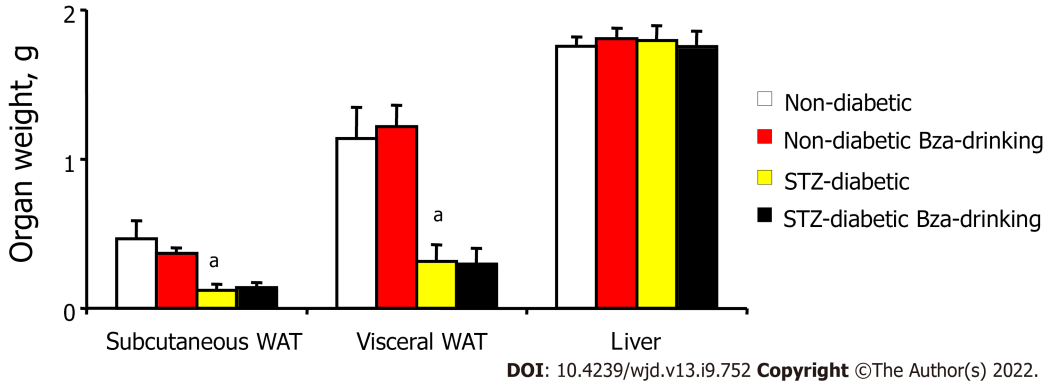

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Figure 3 Lack of influence of Bza supplementation on organ weight in normoglycemic and STZ-induced diabetic mice. Mean ± SEM of the wet weight of subcutaneous or visceral white adipose tissues, and of liver for eight males in each group. Different from nondiabetic mice at: $^{a}P < 0.001$. No significant difference was found between Bza-drinking and respective controls. STZ: Streptozotocin; Bza: Benzylamine; WAT: White adipose tissues.

In contrast, the weight of the liver was identical in the four experimental groups (Figure 3). However, when liver mass was expressed as ratio to body weight, the difference that appeared between diabetic and nondiabetic animals was opposite to that of the adiposomatic index. The liver represented 5.3 ± 0.2% of body mass in both STZ diabetic and STZ diabetic Bza-drinking mice (NS, n = 8). This proportion was smaller in nondiabetic mice (4.2 ± 0.1%, $P < 0.001$), even after Bza drinking (4.5 ± 0.2%).

These observations indicated that the STZ-induced diabetic mice did not normalize their reduced fat deposition and body weight gain after Bza supplementation, in spite of partial recovery of their altered food intake. Moreover, Bza supplementation was not efficient in normalizing the altered blood glucose control or relative hepatomegaly of the STZ mice, although limiting polydipsia. We have previously proposed that Bza oxidation occurring in the hypertrophied WAT of obese and diabetic $db^{-/-}$ mice supports its insulin-like *in vitro* effects by facilitating glucose utilization in adipocytes and contributes to its antihyperglycemic action[1]. Therefore, such *in vitro* effects were examined.

Effects of insulin and Bza on glucose transport in mouse adipocytes

Unfortunately, the WAT atrophy of the STZ diabetic mice did not allow the preparation of sufficient biological material for exploring the activation of 2-DG uptake in functional adipocytes from diabetic and Bza-drinking diabetic mice. There was only a pool of around 400 mg of WAT dissected from different anatomical locations in each STZ mouse, while 1–2 g was removed from each nondiabetic mouse. Consequently, sufficient adipocytes could be isolated from the latter samples only, and the subsequent hexose uptake assays were performed with adipocyte preparations that contained 18.0 ± 2.8 and 19.0 ± 2.5 mg lipid/400 µL in normoglycemic Bza-drinking and control mice, respectively. Thus, Figure 4A shows insulin stimulation of 2-DG uptake in nondiabetic mice only. As expected, insulin dose-dependently activated hexose uptake in adipocytes from control mice, and a tendency to improve insulin maximal effect was detected in Bza-drinking mice. EC_{50} values of insulin were 0.4 and 2.3 nmol/L for Bza-drinking and control mice, respectively, without showing a significant difference between them. Figure 4B indicates that 0.1 mmol/L benzylamine was capable of reproducing one-third of the maximal insulin stimulation, in a manner that was blunted by the amine oxidase inhibitor phenelzine, which was inactive on basal or insulin-stimulated hexose uptake. The amine-oxidase-dependent insulin-like effect of 0.1 mmol/L Bza was similar in control and Bza-drinking nondiabetic mice. There was no influence of oral Bza supplementation on the capacity of phenelzine to inhibit *in vitro* the insulin-like action of the amine (Figure 4B).

Oxidation of Bza in thawed preparations of adipose tissues

Amine oxidase activity was determined in homogenates from thawed WAT samples by measuring their capacity to oxidize 0.1 mmol/L [^{14}C]-Bza. When expressed as nmol amine oxidized/mg protein/min, the activity was limited in WAT from STZ diabetic mice compared to normoglycemic ones, whether in the control or Bza-drinking groups (Figure 5). The reduced amount of WAT and its limited amine oxidase activity did not argue for a strong contribution of fat stores to the biotransformation of the Bza ingested by STZ diabetic mice.

DISCUSSION

At the first glance, the lack of antihyperglycemic effect of Bza drinking described here in STZ diabetic mice contrasts with its antidiabetic action observed in obese and diabetic *db*$^{-/-}$ mice[1]. As discussed below, all these findings converge to propose that the difference in Bza-drinking efficiency between the models of type 1 and type 2 diabetes is not related to insulin deficiency *versus* resistance, but rather to a difference in adiposity between the murine models.

Alongside bearing dramatically larger fat depots than their lean counterparts, the obese and diabetic *db*$^{-/-}$ mice also possess higher levels of SSAO activity in their fat cells[1,32]. Thus, the antihyperglycemic effect of oral Bza reported for *db*$^{-/-}$ mice, and not for their lean littermates, could be related to the elevated amine oxidase activity found in the hypertrophied WAT of obese and diabetic animals[1]. In contrast, STZ diabetic rats exhibit lower monoamine oxidase (MAO) and SSAO activities in WAT than their normoglycemic controls[18]. The lack of antihyperglycemic effect of Bza supplementation in STZ mice reported here resembles the weak antidiabetic effect of prolonged administration of tyramine in STZ rats[18]. Tyramine, which is a substrate of both MAO and SSAO, can limit the hyperglycemic responses to a glucose load during a glucose tolerance test but cannot normalize the elevated fasting blood levels of these insulin-deficient rats. Tyramine or Bza can lower the elevated blood glucose of STZ-induced diabetic rats only when combined with vanadium[10,18,33].

Particular attention has been paid to studying the potential antidiabetic effects of amines alone since the synergism between vanadium and biogenic amines on the activation of glucose transport does not work well in human adipocytes[9,27]. Moreover, the potential antidiabetic use of vanadium derivatives is still limited by toxicological aspects. Several observations suggest that the beneficial effects of dietary amines on glucose handling in diabetic rodents (even when not combined with vanadium) rely upon the amount of SSAO present in WAT. The supplementation of drinking water with 0.4% methylamine (another SSAO substrate) has been reported to increase epididymal WAT mass and to improve glucose tolerance in transgenic mice overexpressing a human form of SSAO/VAP-1, while it is inefficient in nontransgenic mice[34]. Oral Bza also improves glucose handling in high-fat diet fed mice, characterized by increased adiposity[22]. Here, we suppose that it is the lipoatrophy of STZ diabetic mice (and not their lack of insulin) that prevented the occurrence of an antihyperglycemic action of Bza.

The sole beneficial effect of Bza drinking seen in the STZ diabetic mice was an almost total recovery of their characteristic hyperphagic and polydipsic behavior[31]. It could be supposed that urinary glucose leak of STZ mice was partially rescued by Bza drinking. Unfortunately, individual metabolic cages were not available for this study and we could not determine daily urine emission or glucosuria. However, water intake reduction occurred without correction of hyperglycemia. This indicated that renal glucose leak, if any, was not sufficiently rescued by Bza drinking to influence the overall glucose homeostasis, while this was the case for *db*$^{-/-}$ mice[1]. Food intake was also reduced in Bza-drinking STZ diabetic mice, but without notable decrease in body weight gain. Thus, food efficiency was increased by Bza drinking.

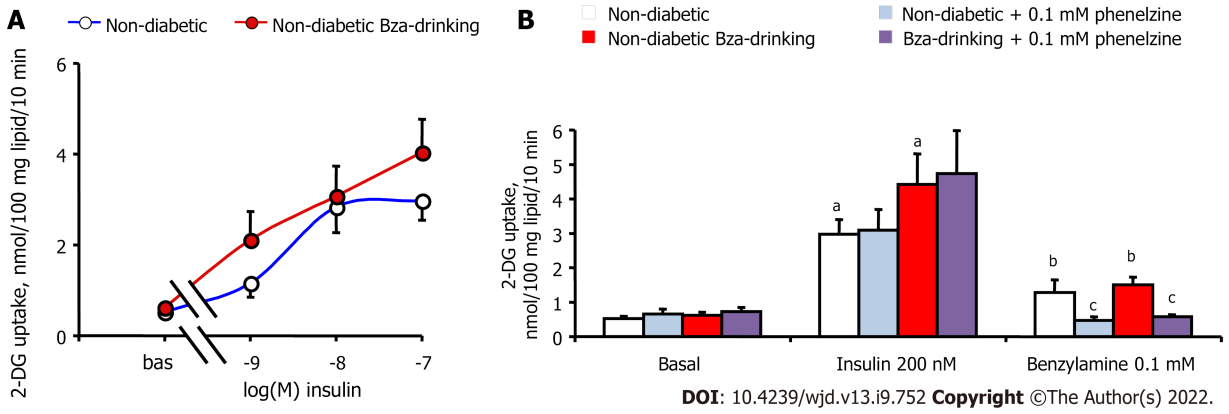

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Figure 4 Direct stimulation by insulin and Bza of hexose uptake in adipocytes: lack of influence of Bza supplementation and *in vitro* inhibition by phenelzine. A: Radiolabeled 2-deoxyglucose (2-DG) uptake was determined in basal condition or in response to increasing doses of insulin in adipocytes from nondiabetic mice of the water-drinking (open circles) or Bza-drinking (red circles) group, while it could not be determined in diabetic mice due to the scarcity of adipocytes isolated from their emaciated fat depots, in both control and Bza-drinking groups. Mean ± SEM of eight adipocyte preparations. B: 2-DG uptake was determined after 45 min incubation without (basal) or with 200 nmol/L insulin and 0.1 mmol/L Bza. The stimulated hexose uptake was significantly different from basal at: $^{a}P < 0.001$; $^{b}P < 0.01$. Phenelzine was added at 0.1 mmol/L in the incubation medium of adipocytes from control nondiabetic mice (blue columns) or from Bza-drinking nondiabetic mice (purple columns). Phenelzine inhibited significantly Bza-induced hexose uptake at: $^{c}P < 0.01$. STZ: Streptozotocin; Bza: Benzylamine; WAT: White adipose tissues.

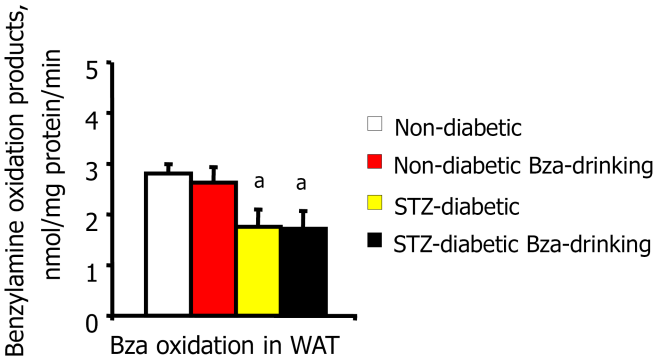

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Figure 5 Bza oxidation in adipose tissue of normoglycemic and STZ-induced diabetic mice: lack of influence of dietary Bza consumption. Radiolabeled Bza was present at 0.1 mmol/L during 30 min incubation at 37°C with WAT homogenates from nondiabetic (white columns), nondiabetic Bza-drinking (red columns), STZ diabetic (yellow columns), STZ diabetic Bza-drinking (black columns). Mean ± SEM of eight determinations for nondiabetic mice and four determinations for lipoatrophic STZ diabetic mice. Different from nondiabetic at: $^{a}P < 0.02$. No significant influence of Bza-treatment was detected. STZ: Streptozotocin; Bza: Benzylamine; WAT: White adipose tissues.

However, we cannot propose any underlying mechanism for this effect.

Indeed, it cannot be excluded that mechanisms other than oxidation by amine oxidases might be involved in the *in vivo* effect of Bza on food and water intake. Raimondi and coworkers have reported that Bza, like methylamine, rapidly induces hypophagia in mice *via* a modulation of neuronal channels, which is reinforced by SSAO inhibition[35,36]. This suggests that adipose SSAO is likely not the sole target of ingested Bza. Regarding activation of glucose uptake in adipocytes, the effect of Bza is impaired when its oxidation by SSAO is blocked. Surprisingly, the opposite occurred regarding its central effects on food and water intake. When Bza degradation by SSAO is blocked, its half-life is increased and its capacity to modulate the neuronal channels depicted by the group of Raimondi is improved[35,36]. Since there is practically no WAT in the STZ-diabetic mice, and since they have little adipose SSAO, we propose that the limitation of hyperphagia and polydipsia observed in these animals is likely due to a central effect distinct from oxidation by peripheral tissues.

Although the liver is another of the organs reached by ingested Bza, it is not a major site for its biotransformation or detoxification because Bza is metabolized to only a small extent by hepatic subcellular fractions, as observed by Mutlib *et al* [37]. By contrast, these authors reported that, when orally given to rats, Bza undergoes oxidative deamination and generates benzaldehyde, then hippuric acid, which is the major metabolite. These authors also observed that Bza was fairly stable in rat plasma despite of the presence of a soluble form of SSAO. Although circulating SSAO activity is known to

increase with diabetes[18,38-40], it is low when compared to the levels of SSAO found in WAT[1]. A putative mediation of the amine effects *via* modulation of insulin secretion can be ruled out because, in another model of insulin-deficient diabetes, the alloxan-injected rat, oral administration of tyramine reduced the hyperglycemia by 35%–43% in a manner that was more dependent on insulin-like than on insulin-releasing actions[41].

A limitation of the study was that insulin plasma levels were not determined throughout the treatment since such measurements were performed only at the beginning. However, since circulating insulin was dramatically decreased by STZ challenge, and since the overt hyperglycemia was not corrected by Bza drinking, it was hypothesized that pancreatic injury was not recovered. The hyperinsulinemic levels of the insulin-resistant $db^{-/-}$ mice remained unchanged after Bza supplementation[1]. Similarly, no change in plasma insulin was found in the $db^{+/+}$ lean control after Bza drinking. Nonetheless, it has been reported that methylamine (another SSAO substrate) limits the insulin degradation by adipocytes[42]. If one supposes that increasing the ability of insulin to stimulate glucose transport is one of the mechanisms involved in the antidiabetic effect of Bza, this can explain why Bza was active in insulin-resistant but not in insulin-deficient diabetes models. Such a paradigm of insulin-sensitizer capacity might provide an alternative to our interpretations based on the necessary abundance of SSAO and WAT to support peripheral glucose disposal. However, it requires to be demonstrated by further investigations, while we report in the current study that Bza alone activated 2-DG uptake in adipocytes, being therefore able to act as an insulin mimicker even in the absence of insulin.

Whether the *in vitro* SSAO-mediated insulin-like effect of Bza is solely responsible for the antihyperglycemic effect of Bza drinking is far from being demonstrated here. However, this assumption agrees with the conclusions of independent studies showing that treatment of diabetic rodents with SSAO inhibitors prevents diabetic complications but is not antihyperglycemic at all[43-45]. All these observations bring evidence that adipose cells are predominantly involved in Bza oxidation, as a consequence of their high SSAO expression[3], although they do not rule out other concomitant mechanisms.

We designed the current study to achieve a similar daily amount of Bza ingested by the STZ diabetic mice to that ingested by the obese and type 2 diabetic $db^{-/-}$ mice[1]. The results showed that such an objective was reached. However, similar amine intake did not result in a similar beneficial influence on glucose disposal in the two models. In the STZ diabetic mice, the lipoatrophy and lower richness of WAT in amine oxidase activity gave less probability for an adipocyte-dependent metabolism of the ingested amine and subsequent insulin-like actions. Another apparent weakness of the present study was that the nondiabetic Swiss mice did not ingest the same daily amount of Bza than those subjected to the STZ diabetogenic challenge. Our experiments showed that the polydipsia of the STZ diabetic mice was early rescued, after the first week of Bza supplementation. They also showed that, among the Bza-drinking groups, the accumulated fluid intake of the STZ diabetic mice was about twice that of the normoglycemic mice. It could be easily justified *post hoc* that, considering the initial polydipsia of diabetic mice, it would have been preferable to double the Bza concentration in the solution given to the Bza-drinking nondiabetic group. Hence, it cannot be excluded that such a high dose of Bza would have reduced liquid consumption in the nondiabetic mice also. By assumption, such an adverse effect on liquid consumption remains unlikely since, as with other organic amines, Bza has a taste varying from almond to fish waste[46], which is not supposed to be repellent for rodents. In reality, achieving exactly the same oral dose of Bza for diabetic and nondiabetic animals would have required weekly pair-adjustments, which are difficult to achieve, and would not have yielded more information about the mechanisms of action. The unchanged lipoatrophy, together with the early recovery of polydipsia in the Bza-drinking group, converge to indicate that the antipolydipsic effect of the amine is mediated by a central effect, distinct from that observed in adipocytes.

The *in vitro* insulin-like effect of submillimolar dose of Bza on glucose transport in adipocytes, and its blockade by phenelzine, reinforced our hypothesis of enhancement of peripheral glucose disposal, although it could not be evidenced in lipoatrophic Bza-drinking STZ mice. Phenelzine, which is a combined MAO and SSAO inhibitor, was used because both MAO and SSAO substrates mimic insulin-like effects in adipocytes[33]. It blocked Bza-stimulated hexose uptake, but not basal or insulin-stimulated hexose uptake. No resistance to the selective blockade by phenelzine appeared in the fat cells from Bza-drinking nondiabetic mice, indicating that continuous supplementation with the substrate did not dramatically downregulate the amine oxidase activities. These hexose uptake assays, which could be performed on nondiabetic mice only, confirmed that, even in the absence of insulin, Bza oxidation activates hexose uptake in adipocytes from Swiss white mice as well as in other rodents[43]. According to the literature, the increase of glucose transport by SSAO activation is limited to adipocytes, and only rare reports have extended this hydrogen-peroxide-dependent insulin-like action to other cell types[47]. Unfortunately, the insufficient number of adipocytes isolated from the atrophied WAT of STZ mice hampered the verification of glucose transport responsiveness to insulin and Bza in the type 1 diabetic state. Even if such insulin mimicry also occurred in adipocytes from insulin-deficient mice, it was too limited to modify the glucose handling, when considering the low mass of WAT, as attested by the significantly lower adiposomatic index found in STZ-treated mice. The limited oxidative metabolism of Bza found in WAT of STZ mice was likely unable to contribute to a replenishment of the atrophied fat

depots *via* the increase of glucose utilization demonstrated in adipocytes of the normoglycemic controls.

Being poorly biotransformed by the limited fat stores of STZ diabetic mice, the ingested Bza could not increase glucose entry in adipocytes and thereby did not contribute to glucose disposal. We presume that such a lack of Bza action explains how its consumption did not decrease elevated blood glucose. Such inefficiency does not preclude future improvements of the antidiabetic therapeutic applications of other amine substrates. However, our findings limit the relevance of Bza consumption to alleviate the complications of type 1 diabetes, especially when accompanied with lipoatrophy. Nevertheless, Bza and its derivatives remain potential antihyperglycemic agents since a recent integrated network pharmacology analysis has revealed that Bza derivatives contribute to the anti-insulin resistance effects of *Moringa oleifera*[48], one of the most potent antidiabetic medicinal plants[30,49,50].

CONCLUSION

Although Bza drinking is devoid of beneficial *in vivo* effects on the type 1 diabetes at doses that limit the onset of type 2 diabetes in genetically obese $db^{-/-}$ mice, the present findings reinforce the hypothesis that oxidation of Bza at the level of adipocytes contributes to peripheral glucose uptake and improves glucose homeostasis. When no sufficient WAT is present (in STZ diabetic mice), the antihyperglycemic effect of Bza is hampered. In contrast, when Bza can be readily oxidized in WAT, it improves glucose tolerance at the expense of an enlargement of fat stores (in $db^{-/-}$ mice). The *in vitro* experiments confirmed the capacity of submillimolar doses of Bza to activate glucose transport in adipocytes. They also show that such SSAO-dependent insulin mimicry is not altered by chronic administration of the substrate.

ARTICLE HIGHLIGHTS

Research background

Oral administration of benzylamine (Bza) exerts antihyperglycemic effects in obese and diabetic rodent models. This effect has been proposed to depend on the insulin-like action of Bza in adipose cells. The amine oxidation catalyzed by amine oxidases abundantly present in adipocytes generates hydrogen peroxide, which activates glucose transport.

Research motivation

To extrapolate the potential antihyperglycemic properties of Bza found in obese and diabetic models to the treatment of insulin-deficient type 1 diabetic states. Bza administration might facilitate glucose utilization to increase lipogenic and adipogenic activities in the adipose tissue and thereby improve glucose disposal even in the absence of insulin.

Research objectives

To evaluate the impact of Bza supplementation on hyperglycemia, polydipsia and hyperphagia in type 1 diabetic mouse, and to demonstrate that Bza metabolism by adipose tissue supports these antidiabetic effects.

Research methods

Bza solution (5 g/L, Bza-drinking) replaced drinking water in streptozotocin (STZ)-induced, insulin-deficient diabetic mice. Similar comparison between control and Bza-drinking groups was performed in normoglycemic mice. Nonfasting blood glucose, water and food intake were periodically recorded in the four groups. Adiposity was determined at the end of a 24-d treatment. Glucose transport in freshly isolated adipocytes was assessed *ex vivo* by determining the uptake of the nonmetabolizable radiolabeled 2-deoxyglucose.

Research results

Chronic Bza intake did not normalize hyperglycemia in STZ diabetic mice, despite it alleviating excessive water and food consumption. Bza intake had no effect on the limited body weight of the STZ diabetic mice and could not restore their dramatically reduced adipose tissue mass. In normoglycemic mice, the Bza-drinking group did not show altered body weight, or food or water consumption. However, when directly given *in vitro* to adipocytes isolated from nondiabetic mice, Bza was efficient in activating glucose uptake in both control and Bza-drinking groups.

Research conclusions

The capacity of Bza supplementation to reduce hyperglycemia, previously reported in obese and diabetic rodents, was not detectable in the emaciated and insulin-deficient STZ diabetic mice. However, the capacity of Bza to activate glucose transport in adipocytes was confirmed in nonobese, nondiabetic

mice. It is likely that the adipose tissue atrophy induced by STZ challenge hampered the lipogenic and adipogenic action of Bza in this severe model of lipoatrophic, insulin-deficient diabetes.

Research perspectives

The current findings and their interpretations considerably limit the field of applications of oral Bza since this molecule did not work as an antidiabetic agent in rodents with reduced adiposity, as it is the case in type 1 STZ diabetic and lipoatrophic mice. Nevertheless, since SSAO substrates exhibit a direct action on glucose handling by fat cells, they still have potential interest for therapeutic use to combat other diabetic states.

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FOOTNOTES

Author contributions: Carpéné C designed the studies, isolated cells for *in vitro* experiments, reviewed the literature, designed the figures, wrote and revised the manuscript; Mercader Barceló J performed animal treatments, non-invasive and *ex vivo* explorations, Stiliyanov Atanasov K was involved in data mining, Les F contributed to statistical analysis, literature review and revised the manuscript.

Institutional review board statement: The study was approved by the I2MC Institutional Review Board: Institut des maladies métaboliques et cadiovasculaires (http://www.i2mc.inserm.fr/accueil).

Institutional animal care and use committee statement: Mice were housed and manipulated according to the INSERM guidelines and European Directive 2010/63/UE by competent and expert technicians or researchers in animal care facilities with agreement number A 31 555 011. The experimental protocol was approved by the local ethical committee CREFRE.

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REFERENCES

1 **Iffiú-Soltesz Z**, Wanecq E, Tóthfalusi L, Szökő É, Carpéné C. Oral Supplementation with Benzylamine Delays the Onset of Diabetes in Obese and Diabetic db-/- Mice. *Nutrients* 2021; **13** [PMID: 34444782 DOI: 10.3390/nu13082622]
2 **Moldes M**, Fève B, Pairault J. Molecular cloning of a major mRNA species in murine 3T3 adipocyte lineage. differentiation-dependent expression, regulation, and identification as semicarbazide-sensitive amine oxidase. *J Biol Chem* 1999; **274**: 9515-9523 [PMID: 10092636 DOI: 10.1074/jbc.274.14.9515]

3 **Shen SH**, Wertz DL, Klinman JP. Implication for functions of the ectopic adipocyte copper amine oxidase (AOC3) from purified enzyme and cell-based kinetic studies. *PLoS One* 2012; **7**: e29270 [PMID: 22238597 DOI: 10.1371/journal.pone.0029270]

4 **Olivieri A**, Tipton KF, O'Sullivan J. Characterization of the in vitro binding and inhibition kinetics of primary amine oxidase/vascular adhesion protein-1 by glucosamine. *Biochim Biophys Acta* 2012; **1820**: 482-487 [PMID: 22202180 DOI: 10.1016/j.bbagen.2011.12.009]

5 **Romauch M**. Zinc-α2-glycoprotein as an inhibitor of amine oxidase copper-containing 3. *Open Biol* 2020; **10**: 190035 [PMID: 32315567 DOI: 10.1098/rsob.190035]

6 **Salmi M**, Jalkanen S. Vascular Adhesion Protein-1: A Cell Surface Amine Oxidase in Translation. *Antioxid Redox Signal* 2019; **30**: 314-332 [PMID: 29065711 DOI: 10.1089/ars.2017.7418]

7 **Czech MP**. Differential effects of sulfhydryl reagents on activation and deactivation of the fat cell hexose transport system. *J Biol Chem* 1976; **251**: 1164-1170 [PMID: 1249070]

8 **Fontana E**, Boucher J, Marti L, Lizcano JM, Testar X, Zorzano A, Carpéné C. Amine oxidase substrates mimic several of the insulin effects on adipocyte differentiation in 3T3 F442A cells. *Biochem J* 2001; **356**: 769-777 [PMID: 11389684 DOI: 10.1042/0264-6021:3560769]

9 **Morin N**, Lizcano JM, Fontana E, Marti L, Smih F, Rouet P, Prévot D, Zorzano A, Unzeta M, Carpéné C. Semicarbazide-sensitive amine oxidase substrates stimulate glucose transport and inhibit lipolysis in human adipocytes. *J Pharmacol Exp Ther* 2001; **297**: 563-572 [PMID: 11303044]

10 **Marti L**, Abella A, Carpéné C, Palacín M, Testar X, Zorzano A. Combined treatment with benzylamine and low dosages of vanadate enhances glucose tolerance and reduces hyperglycemia in streptozotocin-induced diabetic rats. *Diabetes* 2001; **50**: 2061-2068 [PMID: 11522672 DOI: 10.2337/diabetes.50.9.2061]

11 **Mercier N**, Moldes M, El Hadri K, Fève B. Semicarbazide-sensitive amine oxidase activation promotes adipose conversion of 3T3-L1 cells. *Biochem J* 2001; **358**: 335-342 [PMID: 11513731 DOI: 10.1042/0264-6021:3580335]

12 **Yang H**, Ralle M, Wolfgang MJ, Dhawan N, Burkhead JL, Rodriguez S, Kaplan JH, Wong GW, Haughey N, Lutsenko S. Copper-dependent amino oxidase 3 governs selection of metabolic fuels in adipocytes. *PLoS Biol* 2018; **16**: e2006519 [PMID: 30199530 DOI: 10.1371/journal.pbio.2006519]

13 **Paget MB**, Murray HE, Bailey CJ, Downing R. From insulin injections to islet transplantation: An overview of the journey. *Diabetes Obes Metab* 2022; **24** Suppl 1: 5-16 [PMID: 34431589 DOI: 10.1111/dom.14526]

14 **Khalivulla SI**, Mohammed A, Mallikarjuna K. Novel Phytochemical Constituents and their Potential to Manage Diabetes. *Curr Pharm Des* 2021; **27**: 775-788 [PMID: 33355047 DOI: 10.2174/1381612826666201222154159]

15 **Fujikawa T**, Chuang JC, Sakata I, Ramadori G, Coppari R. Leptin therapy improves insulin-deficient type 1 diabetes by CNS-dependent mechanisms in mice. *Proc Natl Acad Sci U S A* 2010; **107**: 17391-17396 [PMID: 20855609 DOI: 10.1073/pnas.1008025107]

16 **Savage DB**. Mouse models of inherited lipodystrophy. *Dis Model Mech* 2009; **2**: 554-562 [PMID: 19892886 DOI: 10.1242/dmm.002907]

17 **Gao H**, Guo Y, Yan Q, Yang W, Li R, Lin S, Bai X, Liu C, Chen D, Cao H, Xiao G. Lipoatrophy and metabolic disturbance in mice with adipose-specific deletion of kindlin-2. *JCI Insight* 2019; **4** [PMID: 31292295 DOI: 10.1172/jci.insight.128405]

18 **Visentin V**, Bour S, Boucher J, Prévot D, Valet P, Ordener C, Parini A, Carpéné C. Glucose handling in streptozotocin-induced diabetic rats is improved by tyramine but not by the amine oxidase inhibitor semicarbazide. *Eur J Pharmacol* 2005; **522**: 139-146 [PMID: 16202994 DOI: 10.1016/j.ejphar.2005.08.051]

19 **Gunawardana SC**, Piston DW. Reversal of type 1 diabetes in mice by brown adipose tissue transplant. *Diabetes* 2012; **61**: 674-682 [PMID: 22315305 DOI: 10.2337/db11-0510]

20 **Conway B**, Miller RG, Costacou T, Fried L, Kelsey S, Evans RW, Orchard TJ. Adiposity and mortality in type 1 diabetes. *Int J Obes (Lond)* 2009; **33**: 796-805 [PMID: 19451912 DOI: 10.1038/ijo.2009.75]

21 **Dias I**, Pinheiro D, Ribeiro Silva K, Stumbo AC, Thole A, Cortez E, de Carvalho L, Carvalho SN. Secretome effect of adipose tissue-derived stem cells cultured two-dimensionally and three-dimensionally in mice with streptozocin induced type 1 diabetes. *Curr Res Pharmacol Drug Discov* 2021; **2**: 100069 [PMID: 34988430 DOI: 10.1016/j.crphar.2021.100069]

22 **Iffiú-Soltész Z**, Wanecq E, Lomba A, Portillo MP, Pellati F, Szöko E, Bour S, Woodley J, Milagro FI, Alfredo Martinez J, Valet P, Carpéné C. Chronic benzylamine administration in the drinking water improves glucose tolerance, reduces body weight gain and circulating cholesterol in high-fat diet-fed mice. *Pharmacol Res* 2010; **61**: 355-363 [PMID: 20045461 DOI: 10.1016/j.phrs.2009.12.014]

23 **Yraola F**, García-Vicente S, Marti L, Albericio F, Zorzano A, Royo M. Understanding the mechanism of action of the novel SSAO substrate (C7NH10)6(V10O28).2H2O, a prodrug of peroxovanadate insulin mimetics. *Chem Biol Drug Des* 2007; **69**: 423-428 [PMID: 17581236 DOI: 10.1111/j.1747-0285.2007.00516.x]

24 **Shechter Y**, Karlish SJ. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. *Nature* 1980; **284**: 556-558 [PMID: 6988725 DOI: 10.1038/284556a0]

25 **Srivastava AK**, Mehdi MZ. Insulino-mimetic and anti-diabetic effects of vanadium compounds. *Diabet Med* 2005; **22**: 2-13 [PMID: 15606684 DOI: 10.1111/j.1464-5491.2004.01381.x]

26 **Zorzano A**, Palacín M, Marti L, García-Vicente S. Arylalkylamine vanadium salts as new anti-diabetic compounds. *J Inorg Biochem* 2009; **103**: 559-566 [PMID: 19246098 DOI: 10.1016/j.jinorgbio.2009.01.015]

27 **Carpéné C**, Boulet N, Grolleau JL, Morin N. High doses of catecholamines activate glucose transport in human adipocytes independently from adrenoceptor stimulation or vanadium addition. *World J Diabetes* 2022; **13**: 37-53 [PMID: 35070058 DOI: 10.4239/wjd.v13.i1.37]

28 **Rancoule C**, Attané C, Grès S, Fournel A, Dusaulcy R, Bertrand C, Vinel C, Tréguer K, Prentki M, Valet P, Saulnier-Blache JS. Lysophosphatidic acid impairs glucose homeostasis and inhibits insulin secretion in high-fat diet obese mice. *Diabetologia* 2013; **56**: 1394-1402 [PMID: 23508306 DOI: 10.1007/s00125-013-2891-3]

29 **Kilkenny C**, Browne W, Cuthill IC, Emerson M, Altman DG; NC3Rs Reporting Guidelines Working Group. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol* 2010; **160**: 1577-1579 [PMID: 20649561

DOI: 10.1111/j.1476-5381.2010.00872.x]

30 **Jaiswal D**, Kumar Rai P, Kumar A, Mehta S, Watal G. Effect of Moringa oleifera Lam. leaves aqueous extract therapy on hyperglycemic rats. *J Ethnopharmacol* 2009; **123**: 392-396 [PMID: 19501271 DOI: 10.1016/j.jep.2009.03.036]

31 **Granneman JG**, Stricker EM. Food intake and gastric emptying in rats with streptozotocin-induced diabetes. *Am J Physiol* 1984; **247**: R1054-R1061 [PMID: 6239554 DOI: 10.1152/ajpregu.1984.247.6.R1054]

32 **Cioni L**, De Siena G, Ghelardini C, Sernissi O, Alfarano C, Pirisino R, Raimondi L. Activity and expression of semicarbazide-sensitive benzylamine oxidase in a rodent model of diabetes: interactive effects with methylamine and alpha-aminoguanidine. *Eur J Pharmacol* 2006; **529**: 179-187 [PMID: 16325802 DOI: 10.1016/j.ejphar.2005.10.052]

33 **Marti L**, Morin N, Enrique-Tarancon G, Prevot D, Lafontan M, Testar X, Zorzano A, Carpéné C. Tyramine and vanadate synergistically stimulate glucose transport in rat adipocytes by amine oxidase-dependent generation of hydrogen peroxide. *J Pharmacol Exp Ther* 1998; **285**: 342-349 [PMID: 9536030]

34 **Stolen CM**, Madanat R, Marti L, Kari S, Yegutkin GG, Sariola H, Zorzano A, Jalkanen S. Semicarbazide sensitive amine oxidase overexpression has dual consequences: insulin mimicry and diabetes-like complications. *FASEB J* 2004; **18**: 702-704 [PMID: 14977883 DOI: 10.1096/fj.03-0562fje]

35 **Banchelli G**, Ghelardini C, Raimondi L, Galeotti N, Pirisino R. Selective inhibition of amine oxidases differently potentiate the hypophagic effect of benzylamine in mice. *Eur J Pharmacol* 2001; **413**: 91-99 [PMID: 11173067 DOI: 10.1016/s0014-2999(01)00739-7]

36 **Pirisino R**, Ghelardini C, Banchelli G, Galeotti N, Raimondi L. Methylamine and benzylamine induced hypophagia in mice: modulation by semicarbazide-sensitive benzylamine oxidase inhibitors and aODN towards Kv1.1 channels. *Br J Pharmacol* 2001; **134**: 880-886 [PMID: 11606329 DOI: 10.1038/sj.bjp.0704316]

37 **Mutlib AE**, Dickenson P, Chen SY, Espina RJ, Daniels JS, Gan LS. Bioactivation of benzylamine to reactive intermediates in rodents: formation of glutathione, glutamate, and peptide conjugates. *Chem Res Toxicol* 2002; **15**: 1190-1207 [PMID: 12230413 DOI: 10.1021/tx020063q]

38 **Mészáros Z**, Szombathy T, Raimondi L, Karádi I, Romics L, Magyar K. Elevated serum semicarbazide-sensitive amine oxidase activity in non-insulin-dependent diabetes mellitus: correlation with body mass index and serum triglyceride. *Metabolism* 1999; **48**: 113-117 [PMID: 9920154 DOI: 10.1016/s0026-0495(99)90019-7]

39 **Salmi M**, Stolen C, Jousilahti P, Yegutkin GG, Tapanainen P, Janatuinen T, Knip M, Jalkanen S, Salomaa V. Insulin-regulated increase of soluble vascular adhesion protein-1 in diabetes. *Am J Pathol* 2002; **161**: 2255-2262 [PMID: 12466139 DOI: 10.1016/S0002-9440(10)64501-4]

40 **Januszewski AS**, Mason N, Karschimkus CS, Rowley KG, Best JD, O'Neal DN, Jenkins AJ. Plasma semicarbazide-sensitive amine oxidase activity in type 1 diabetes is related to vascular and renal function but not to glycaemia. *Diab Vasc Dis Res* 2014; **11**: 262-269 [PMID: 24853908 DOI: 10.1177/1479164114532963]

41 **Lino CdS**, Sales TdP, Gomes PB, Amaral JFd, Alexandre FSA, Silveira ER, Ferreira JM, Daniel F. de Sousa, Queiroz MGRd, Sousa FCFd, Brito GAC, Brito SMRC, Viana GSB. Anti-diabetic activity of a fraction from Cissus verticillata and tyramine, its main bioactive constituent, in alloxan-induced diabetic rats. *Am J Pharmacol Toxicol* 2007; **2**: 178-188 [DOI: 10.3844/ajptsp.2007.178.188]

42 **Kahn CR**, Baird KL. Pathways of insulin degradation in isolated adipocytes: evaluation by gel filtration and differential precipitation. *Metabolism* 1985; **34**: 354-363 [PMID: 3884964 DOI: 10.1016/0026-0495(85)90225-2]

43 **Yu PH**, Wang M, Fan H, Deng Y, Gubisne-Haberle D. Involvement of SSAO-mediated deamination in adipose glucose transport and weight gain in obese diabetic KKAy mice. *Am J Physiol Endocrinol Metab* 2004; **286**: E634-E641 [PMID: 14656718 DOI: 10.1152/ajpendo.00272.2003]

44 **Papukashvili D**, Rcheulishvili N, Deng Y. Beneficial Impact of Semicarbazide-Sensitive Amine Oxidase Inhibition on the Potential Cytotoxicity of Creatine Supplementation in Type 2 Diabetes Mellitus. *Molecules* 2020; **25** [PMID: 32349282 DOI: 10.3390/molecules25092029]

45 **Tékus V**, Horváth ÁI, Csekő K, Szabadfi K, Kovács-Valasek A, Dányádi B, Deres L, Halmosi R, Sághy É, Varga ZV, Adeghate E, Kőszegi T, Mátyus P, Gábriel R, Ferdinandy P, Pintér E, Helyes Z. Protective effects of the novel amine-oxidase inhibitor multi-target drug SZV 1287 on streptozotocin-induced beta cell damage and diabetic complications in rats. *Biomed Pharmacother* 2021; **134**: 111105 [PMID: 33338750 DOI: 10.1016/j.biopha.2020.111105]

46 **Olivieri A**, Rico D, Khiari Z, Henehan G, O'Sullivan J, Tipton K. From caffeine to fish waste: amine compounds present in food and drugs and their interactions with primary amine oxidase. *J Neural Transm (Vienna)* 2011; **118**: 1079-1089 [PMID: 21373760 DOI: 10.1007/s00702-011-0611-z]

47 **El Hadri K**, Moldes M, Mercier N, Andreani M, Pairault J, Feve B. Semicarbazide-sensitive amine oxidase in vascular smooth muscle cells: differentiation-dependent expression and role in glucose uptake. *Arterioscler Thromb Vasc Biol* 2002; **22**: 89-94 [PMID: 11788466 DOI: 10.1161/hq0102.101550]

48 **Huang Q**, Liu R, Liu J, Huang Q, Liu S, Jiang Y. Integrated Network Pharmacology Analysis and Experimental Validation to Reveal the Mechanism of Anti-Insulin Resistance Effects of *Moringa oleifera* Seeds. *Drug Des Devel Ther* 2020; **14**: 4069-4084 [PMID: 33116398 DOI: 10.2147/DDDT.S265198]

49 **Kar A**, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *J Ethnopharmacol* 2003; **84**: 105-108 [PMID: 12499084 DOI: 10.1016/s0378-8741(02)00144-7]

50 **Gupta R**, Mathur M, Bajaj VK, Katariya P, Yadav S, Kamal R, Gupta RS. Evaluation of antidiabetic and antioxidant activity of Moringa oleifera in experimental diabetes. *J Diabetes* 2012; **4**: 164-171 [PMID: 22103446 DOI: 10.1111/j.1753-0407.2011.00173.x]

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Role of insulin in pancreatic microcirculatory oxygen profile and bioenergetics

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Abstract

BACKGROUND

The pancreatic islet microcirculation adapts its metabolism to cope with limited oxygen availability and nutrient delivery. In diabetes, the balance between oxygen delivery and consumption is impaired. Insulin has been proven to exert complex actions promoting the maintenance of homeostasis of the pancreas under glucotoxicity.

AIM

To test the hypothesis that insulin administration can improve the integrated pancreatic microcirculatory oxygen profile and bioenergetics.

METHODS

The pancreatic microcirculatory partial oxygen pressure (PO_2), relative hemoglobin (rHb) and hemoglobin oxygen saturation (SO_2) were evaluated in nondiabetic, type 1 diabetes mellitus (T1DM), and insulin-treated mice. A three-dimensional framework was generated to visualize the microcirculatory oxygen profile. Ultrastructural changes in the microvasculature were examined using transmission electron microscopy. An Extracellular Flux Analyzer was used to detect the real-time changes in bioenergetics by measuring the oxygen consumption rate and extracellular acidification rate in islet microvascular endothelial cells (IMECs).

RESULTS

Significantly lower PO_2, rHb, and SO_2 values were observed in T1DM mice than in

nondiabetic controls. Insulin administration ameliorated the streptozotocin-induced decreases in these microcirculatory oxygen parameters and improved the mitochondrial ultrastructural abnormalities in IMECs. Bioenergetic profiling revealed that the IMECs did not have spare respiratory capacity. Insulin-treated IMECs exhibited significantly greater basal respiration than glucotoxicity-exposed IMECs ($P < 0.05$). An energy map revealed increased energetic metabolism in insulin-treated IMECs, with significantly increased ATP production, non-mitochondrial respiration, and oxidative metabolism (all $P < 0.05$). Significant negative correlations were revealed between microcirculatory SO_2 and bioenergetic parameters.

CONCLUSION
Glucotoxicity deteriorates the integrated pancreatic microcirculatory oxygen profile and bioenergetics, but this deterioration can be reversed by insulin administration.

Key Words: Diabetes mellitus; Glucotoxicity; Endothelial cells; Microcirculation; Mitochondria; Bioenergetics

Core Tip: The pancreatic islet microvasculature adapts its metabolism to cope with limited oxygen availability and nutrient delivery. Insulin has been proven to exert complex actions promoting the maintenance of homeostasis under glucotoxicity. Our findings demonstrate that insulin ameliorates the suppression of the integrated microcirculatory oxygen profile in type 1 diabetes mellitus mice and improves mitochondrial ultrastructural abnormalities in islet microvascular endothelial cells (IMECs). Additionally, insulin administration restores glucotoxicity-induced microcirculatory failure by increasing the mitochondrial basal respiration and glycolytic capacity of IMECs.

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INTRODUCTION

The concept of pancreatic islet microcirculation, which is currently under the spotlight[1,2], is responsible for coupling metabolic demands with glucose distribution and oxygen delivery in a manner involving microvascular endothelium-dependent vasodilation. Emerging evidence, including ours, indicates that the integrated pancreatic microcirculation in islets is necessary to maintain endocrine function and is involved in the pathogenesis of diabetes, partially through impairment of microcirculatory blood perfusion[3,4].

As part of a highly specialized microvascular system[5], pancreatic islets are richly vascularized and have 5-10-fold higher blood flow than the exocrine pancreas[6]. Pancreatic islet microvascular endothelial cells (IMECs) are therefore responsible for maintaining substance exchange and contribute to the dynamic regulation of glucose metabolism. Malfunction of IMECs is not only the culprit of deteriorated pancreatic islet microvascular blood perfusion and oxygen supply but also a victim of imbalanced energetic homeostasis.

Metabolic abnormalities in glucose are generally related to alterations in energy metabolism, especially at the onset of diabetes. The main organelle of IMECs responsible for energetic homeostasis is the mitochondrion, which plays a critical role in IMEC survival and death by regulating ATP synthesis through glucose metabolism, ROS generation, and apoptosis[7,8]. Furthermore, the metabolic profile of IMECs links the microcirculatory phenomena to the occurrence of pathological phenotypes. It is therefore important and rational to investigate the metabolic states of IMECs to clarify the microcirculatory pathological changes that occur under glucotoxicity.

Several reports have suggested the involvement of microcirculatory endothelial dysfunction in diabetes. However, knowledge surrounding the bioenergetics of IMECs related to insufficient microcirculatory oxygen is rather limited. We have established a new microcirculatory monitoring approach that integrates pancreatic microcirculatory partial oxygen pressure (PO_2), relative hemoglobin (rHb) and hemoglobin oxygen saturation (SO_2) using a multimodal device[9]. Thus, the purpose of this study was to describe the integrated pancreatic microcirculatory oxygen under glucotoxicity and to determine the impact of insulin on the microcirculatory oxygen and bioenergetic profile of IMECs.

MATERIALS AND METHODS

Animals

BALB/c mice were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (CAMS). The mice were bred and housed at 22 ± 1 °C with 55%-65% humidity under a 12 h:12 h light:dark cycle. The mice were randomly divided into three groups, including a type 1 diabetes mellitus (T1DM) model group, an insulin-treated group and a nondiabetic control group (all n = 3). T1DM was established by intraperitoneal administration of streptozotocin (STZ, 40 mg/kg) into the mice for five consecutive days. A level of blood glucose higher than 200 mg/dL was considered to indicate diabetes. Insulin was subcutaneously injected (1.5 IU/day) into the mice in the insulin-treated group to maintain the blood glucose within the normal range[10]. The animal experiments in this study were permitted by the Laboratory Animal Welfare and Ethics Committee, Institute of Microcirculation, CAMS (China).

Measurements of the microcirculatory oxygen profile

To assess pancreatic microcirculatory oxygen, we employed a multimodal auxiliary microcirculatory monitoring system established with a fiber-optic oxygen sensor (Precision Sensing, Regensburg, Germany) and an Oxygen to See device (LEA Medizintechnik, Giessen, Germany) to determine the SO_2, rHb, and PO_2. After anesthesia, the pancreas was gently exposed by a median abdominal incision, and the microcirculatory oxygen profile, including SO_2, rHb, and PO_2, was subsequently captured. These parameters were measured at three random sites of the pancreas in each mouse.

Establishment of the three-dimensional framework of the microcirculatory oxygen profile

Python (ver 3.7.4) and Apache ECharts (ver 4.2.0-rc.2) were used to generate a three-dimensional framework to visualize the pancreatic microcirculatory oxygen profile. In the 3-D framework, the X-, Y-, and Z-axes represented the time course, microcirculatory oxygen variables, and calculated values of the microcirculatory oxygen profile, respectively. The outliers were adjusted by the box plot algorithm. Additionally, the least common multiple algorithm was used to adjust the time granularity. Min-max normalization was conducted to transform the dimensions of multiple parameters.

Video recording of the microcirculatory oxygen profile

ScreenToGif (version 2.19.3) was used to capture the dynamic 3-D framework. Each video was recorded in an MPEG4 file. The bitrate was 2000 Kbps with a 1920 × 1080 resolution ratio.

Transmission electron microscopy

Ultrastructural changes in the pancreatic islet microvasculature were examined using transmission electron microscopy (TEM). Fresh pancreatic tissue was fixed in 3% glutaraldehyde and 1% osmic acid and then passed through a graded series of dehydration and embedding solutions. Ultrathin sections (70 nm) were made after resin polymerization and uranyl acetate/Lead citrate staining. The samples were examined using a JEM-1400Plus transmission electron microscope (JEOL Ltd., Tokyo, Japan). The mitochondrial ultrastructure of IMECs was assessed.

Cell culture

The IMECs were purchased from ATCC (MS1, Manassas, VA, United States) and routinely cultured in DMEM supplemented with 10% FBS, 5.6 mmol/L glucose, 2% HEPES and 100 U of penicillin–streptomycin (Gibco, Carlsbad, CA, United States). After IMECs grew to confluence, the cells were divided into three groups, the control, glucotoxicity-exposed (HG, 25 mmol/L glucose), and insulin-treated (HG + Ins, 25 mmol/L glucose plus 10^{-8} mol/L insulin[10]) groups, and were treated for 24 h (n = 4 each group).

Bioenergetics assay

To investigate the effects of the high concentration of glucose with or without insulin on the bioenergetics of IMECs, an Extracellular Flux Analyzer (XFe24, Seahorse Bioscience, Billerica, MA, United States) was used to detect the real-time changes in energy pathways by directly probing the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Briefly, IMECs were seeded in XFe24 culture plates at 1×10^4 cells per well. The cells were treated according to the abovementioned grouping for 24 h in DMEM with 0.5% FBS. The medium was subsequently replaced by Seahorse XF assay medium, and the cells were incubated without CO_2 for 1 h until detection.

For the mitochondrial stress test, mitochondrial function was monitored along with the sequential addition of oligomycin, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) and rotenone/antimycin A (all 0.5 μM). Multiple respiratory indexes, including baseline metabolic functions (basal respiration, proton leak, ATP-linked respiration, non-mitochondrial respiration, and oxidative metabolism) and metabolic stress responses (maximum respiratory capacity [MRC] and spare respiratory capacity [SRC]), were analyzed and compared. In addition, an ECAR value was probed to

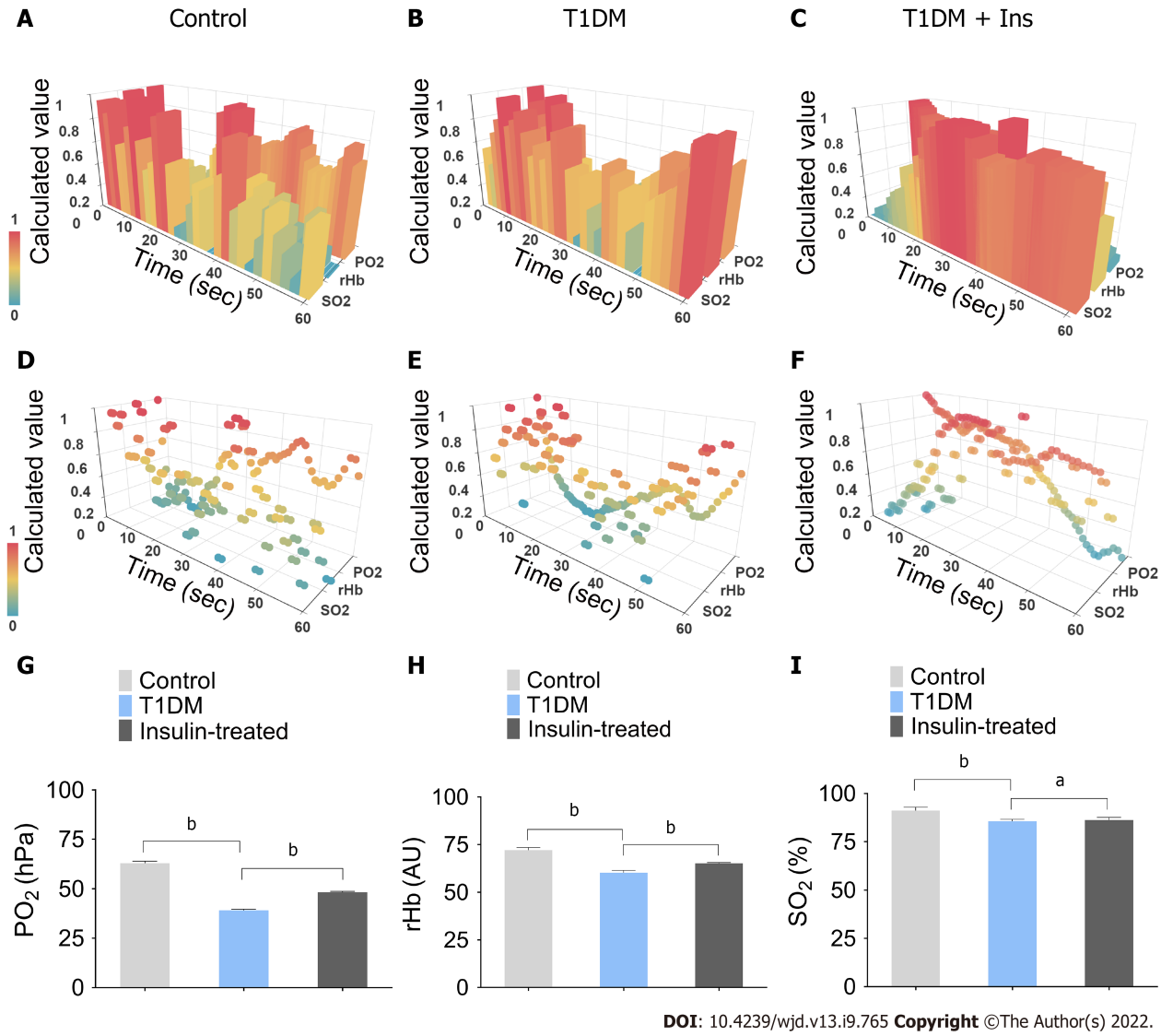

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Figure 1 Integrated pancreatic microcirculatory oxygen profile. A-F: The pancreatic microcirculatory oxygen parameters of control, type 1 diabetes mellitus (T1DM) and insulin-treated mice were captured by probes of O2C and Microx TX3. Python and Apache ECharts were used to generate and visualize the three-dimensional (3-D) module of the integrated pancreatic microcirculatory oxygen profile; G-I: Comparisons of pancreatic microcirculatory oxygen profiles among groups. Partial pressure of oxygen, relative amount of hemoglobin, and hemoglobin oxygen saturation levels in control and T1DM mice with or without insulin administration are illustrated. $^{a}P < 0.05$, $^{b}P < 0.01$. Control, control mice; T1DM, STZ-induced T1DM mice without insulin administration; Insulin-treated, STZ-induced diabetic mice with 1.5 IU administration. T1DM: type 1 diabetes mellitus; Ins: Insulin; SO_2: Hemoglobin oxygen saturation; rHb: Relative amount of hemoglobin; PO_2: Partial pressure of oxygen; O2C: Oxygen to See.

indicate the basal glycolytic function when 10 mmol/L glucose was preadded into the medium before any pharmacological intervention. The ECAR-associated glycolytic capacity was subsequently reached after the injection of oligomycin. In this study, the values of both OCR and ECAR were normalized to 10^4 cells.

Statistical analysis

SPSS software 21.0 (IBM, Armonk, NY) was used to perform the statistical analyses. The data are shown as the mean ± standard error of the mean. The data sets were subjected to one-way ANOVA and post hoc multiple comparisons. A *P* value under 0.05 was considered to indicate statistical significance. In addition, the correlation between the microcirculatory oxygen profile and bioenergetics of IMECs was analyzed by Pearson's method.

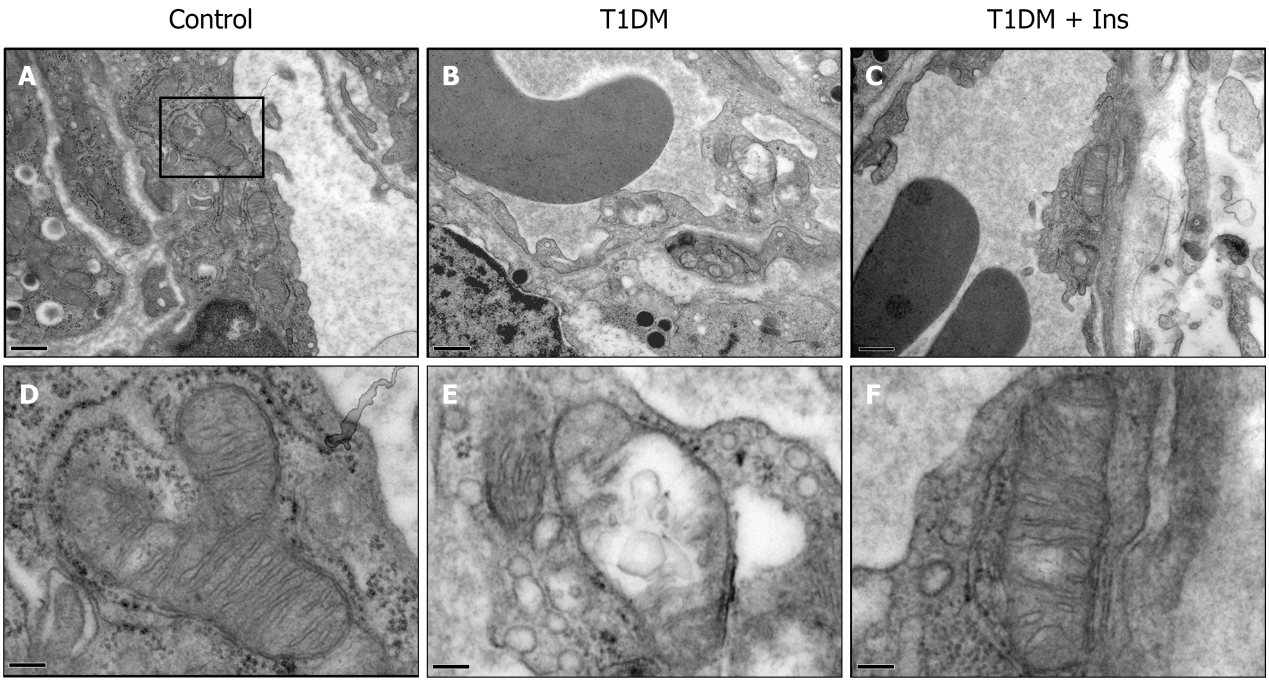

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Figure 2 Glucotoxicity induced ultrastructural damage to mitochondria in islet microvascular endothelial cells. The ultrastructure of pancreatic islet microvascular endothelial cells (IMECs) in the control (A), type 1 diabetes mellitus (T1DM) (B) and insulin-treated groups (C) was revealed by TEM (upper panels, scale bar = 0.5 μm). The ultrastructure of mitochondria in IMECs in the control (D), T1DM (E) and insulin-treated groups (F) is shown in the lower panels. Swollen mitochondria with cristae rupture or disappearance and a transparent matrix were found in T1DM mice. Restored mitochondria were observed after insulin administration (lower panels, scale bar = 2 μm).

RESULTS

Insulin ameliorates the decrease in the integrated microcirculatory oxygen profile

In this study, the efficiency of STZ to induce T1DM mice model was 100%. To analyze the integrated microcirculatory oxygen profile of islet microcirculation, the preprocessed raw data were imported into the common microcirculatory framework. The oscillation of the microcirculatory oxygen profile is shown in histograms of the 3-D module (Figure 1A-C), and the distribution pattern of the microcirculatory oxygen profile was indicated using a scatter plot (Figure 1D-F, Videos 1-6). Loss of microcirculatory oxygen was observed in T1DM mice, which exhibited a significant decrease in PO_2, rHb, and SO_2 compared with nondiabetic controls. Additionally, insulin administration ameliorated the STZ-induced decreases in these microcirculatory oxygen parameters (Figure 1G-I).

Insulin improves the mitochondrial ultrastructural abnormalities in IMECs in T1DM mice

Given that microvessels are responsible for distributing oxygen, we sought to determine whether the mitochondrial ultrastructure of IMECs changes in T1DM mice. TEM revealed the normal architecture of IMECs in the nondiabetic control group, with ovoid nuclei and well-arranged mitochondria in the cytoplasm. In contrast, mice with T1DM showed a narrowed or closed lumen with a contorted and thickened basement membrane, nuclear disaggregation, and mitochondrial swelling, suggesting an impaired ultrastructure of mitochondria in IMECs. The mitochondria in insulin-treated IMECs were restored, with a thin basement membrane, wide capillary lumen, and well-arranged parallel cristae (Figure 2). These data confirm the protective effect of insulin in the microcirculation of T1DM mice.

Effects of glucotoxicity and insulin administration on OCR and ECAR

The tight integration between endothelial metabolism and microcirculatory oxygen transport begs for integrative analysis that spans the cellular scale. We then performed real-time analysis of OCR and ECAR to determine energetic metabolic features in IMECs. The OCR of the IMECs was determined before and after receiving interventions of oligomycin, FCCP, and rotenone/antimycin A. A schematic of the real-time analysis of the IMEC OCR is depicted in Figure 3A. Our data revealed comparable mitochondrial maximal respiration in control, glucotoxicity-exposed, and insulin-treated IMECs, which was not induced after the injection of FCCP (Figure 3B).

Subsequently, to determine whether FCCP concentration is responsible for the evaluation of the MRC, the IMECs were incubated with different concentrations of FCCP (0.125, 0.25, 0.5, 1, and 2 μM) in the control and HG groups. Surprisingly, none of the OCR values exceeded the corresponding basal

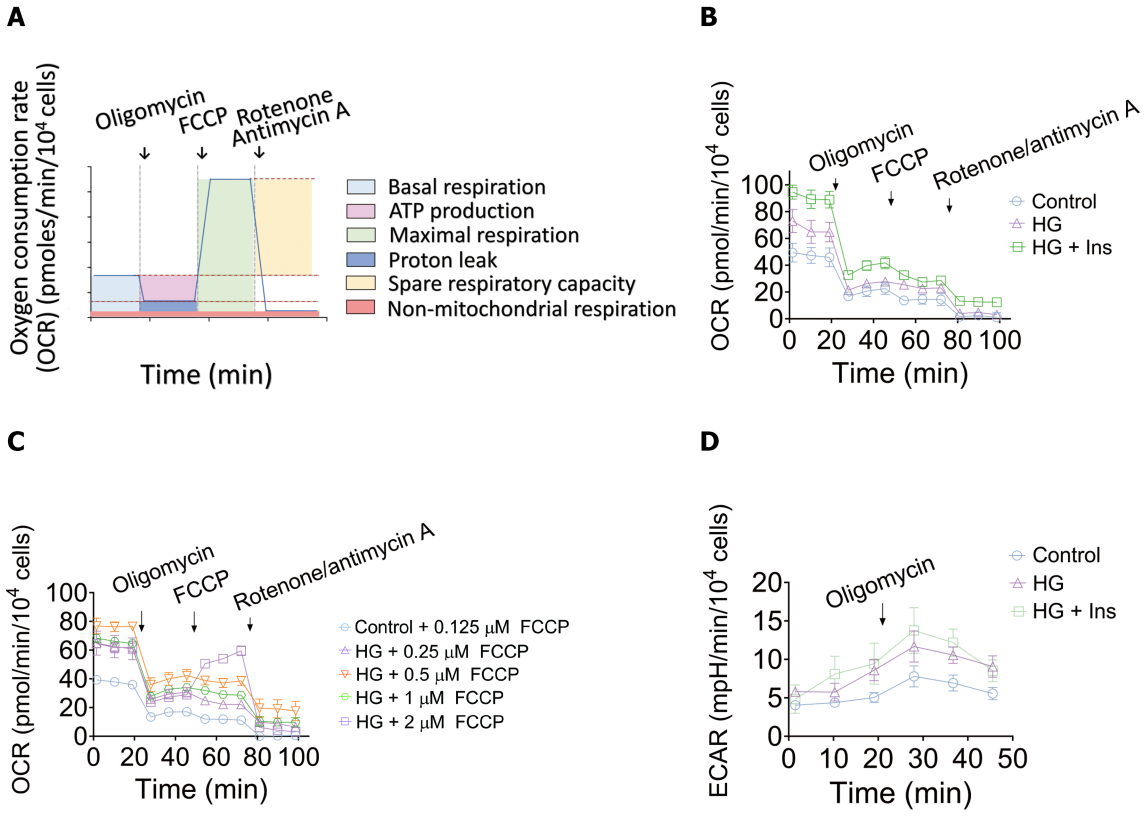

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Figure 3 Characterization of mitochondrial function in the control, glucotoxicity-exposed, and insulin-treated islet microvascular endothelial cell groups. A: Schematic representation of real-time mitochondrial respiration. The parameters of mitochondrial function were measured by kinetic oxygen consumption rate (OCR) analysis, starting from basal detection and after the injection of oligomycin (complex V inhibition), carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP, maximal respiration induction), and rotenone/antimycin A mixture (electron transport chain inhibition). Non-mitochondrial respiration was directly measured. Basal respiration, ATP production, maximal respiration, proton leak, and mitochondrial spare respiratory capacity were then calculated according to the OCR curve; B: Representative kinetic curve of mitochondrial OCR in control, glucotoxicity-exposed islet microvascular endothelial cells (IMECs) (HG), and insulin-treated IMECs (HG + Ins) after sequential injection of oligomycin, FCCP, and rotenone/antimycin A; C: Representative kinetic curve of mitochondrial OCR in control and glucotoxicity-exposed IMECs (HG) after sequential injection of gradient FCCP concentrations; D: Representative kinetic curve of extracellular acidification rate (ECAR) after injection of oligomycin. The peak values of ECAR reflect the glycolytic capacity. The data are presented as the mean ± SEM, $n = 4$ for each group. OCR: Oxygen consumption rate; FCCP: Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; HG: High glucose; Ins: Insulin.

OCR after the FCCP injections, suggesting that the IMECs do not have SRC (Figure 3C). The ECAR values were simultaneously measured to reflect the glycolytic activity of IMECs. After 0.5 μM oligomycin injection, the glycolytic capacity was recorded as the peak value of ECAR (Figure 3D).

Insulin administration increases basal respiration and glycolytic capacity

Insulin-treated IMECs exhibited significantly increased basal respiration in comparison with glucotoxicity-exposed IMECs ($P < 0.05$, Figure 4A). However, there were no significant differences in the basal glycolytic activity among the groups (all $P > 0.05$, Figure 4B). Moreover, an energy map was plotted based on the basal respiration and glycolytic activity in the IMECs. IMECs in the control group were in the quiescent quadrant (lower left). Glucotoxicity-exposed IMECs shifted to the energetic quadrant (upper right), reflecting increased mitochondrial activity. Insulin-administered IMECs were located in the right upper energetic quadrant, revealing more energetic metabolism (Figure 4C), suggesting that insulin increased the glycolytic activity of glucotoxicity-exposed IMECs when needed.

Insulin administration activates oxidative metabolism and alleviates glucotoxicity-induced microcirculatory failure in IMECs

The basal respiration of mitochondria and non-mitochondrial respiration constitute oxidative metabolism in IMECs. Specific mitochondrial and non-mitochondrial functions were subsequently analyzed. ATP production, non-mitochondrial respiration, and oxidative metabolism were significantly increased in insulin-treated IMECs ($P < 0.05$, Figure 5A, D and E). However, proton leak (Figure 5B), coupling efficiency (Figure 5C), and endothelial glycolytic capacity (Figure 5F) were comparable among the groups.

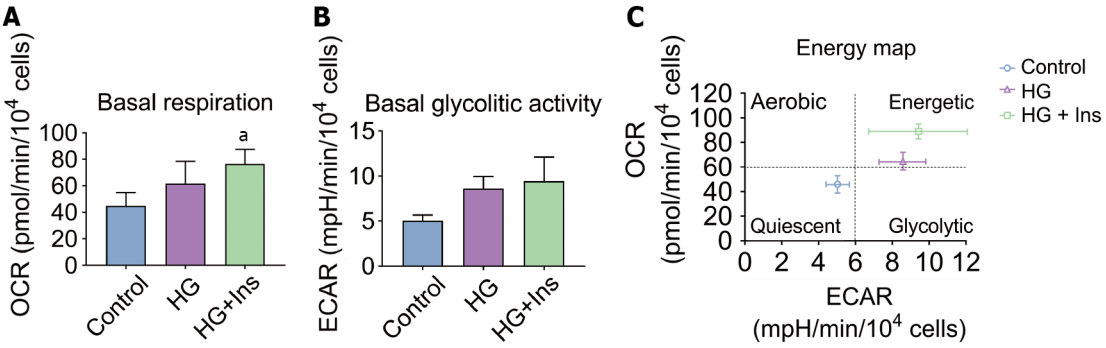

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Figure 4 Basal respiration and glycolytic activity in islet microvascular endothelial cells. The oxygen consumption rate and extracellular acidification rate were measured and compared among control, glucotoxicity-exposed islet microvascular endothelial cells (IMECs) (HG), and IMECs (HG + Ins). A: Basal respiration among groups; B: Basal glycolytic activity among groups; C: Glucotoxicity-exposed and insulin administered IMECs display distinct metabolic phenotypes. The data are presented as the mean ± SEM, $n = 4$ for each group. $^{a}P < 0.05$. OCR: Oxygen consumption rate; ECAR: Extracellular acidification rate; HG: High glucose; Ins: Insulin.

The correlation between the microcirculatory oxygen profile and bioenergetics of IMECs was then analyzed. Significant negative correlations between microcirculatory SO_2 and bioenergetic parameters (ATP production, basal respiration, oxidative metabolism, and non-mitochondrial respiration) were found by Pearson's correlation analysis (Figure 5G). These lines of evidence further confirmed that glucotoxicity in IMECs was related to pancreatic islet microcirculatory failure that could be reversed by insulin administration.

DISCUSSION

The influence of microcirculatory disturbance on the development of diabetes mellitus has been highlighted over decades[11]. However, the current data associated with pancreatic microcirculatory oxygen profiles are deficient. Here, we used a computer algorithm-based common microcirculatory framework to reveal integrated pancreatic microcirculatory oxygen profiles among groups. The existence of microcirculatory hypoxia in T1DM was noted.

Considering islet β cells, rather than IMECs, are specific target of STZ. Therefore, STZ-involved T1DM animal models are useful in elucidating the mechanisms of diabetic microvascular endothelial pathogenesis. IMECs are the key determinants in pancreatic islet microcirculation homeostasis. Blood perfusion and oxygen transport requires the coordinated communication of mitochondria with metabolic demands, which is influenced by a variety of factors (including hypoxia)[12]. Coinciding with the impairment in the microcirculatory oxygen profile, pathological alterations in mitochondrial ultrastructure and other subcellular structures have been observed in IMECs of T1DM mice. Earlier studies have reported that defects in mitochondrial function correlate with mal-matching adenosine triphosphate generation[13,14], which interferes with the bioenergetics of pancreatic islet microcirculation. Treatment with insulin during glucotoxicity exposure resulted in restoration of the ultrastructure of IMECs. Thus, our data suggest that insulin can improve the functional status of pancreatic islet microcirculation.

Metabolic capacity is important for energy regulation and the maintenance of cell survival[15]. In parallel with damage to the ultrastructure of IMECs, biogenetic mechanisms act during glucotoxicity exposure to compensate for the decreased blood perfusion and oxygen distribution. IMECs supplied with insulin increase their basal respiration and ATP production and switch to energetic adaptation. Mitochondria are important organelles for ATP production[16]. Dysfunction of mitochondria is one of the key determinants in the pathogenesis of diabetes[17].

Unexpectedly, our results indicated that maximal respiration of the mitochondria was not induced after injection of FCCP. Multiple factors are associated with FCCP-induced maximal respiration of mitochondria[18]. Therefore, to exclude the effect of FCCP concentration, we subsequently tested five FCCP concentrations, but none caused the basal OCR value to be exceeded, suggesting that the IMECs do not have SRC. In addition to the organ- and tissue-specific nature of microvascular endothelial cells [19], one of the possible explanations is that IMECs generate more than 85% of their ATP through glycolysis[20], which does not require an excessive number of mitochondria to obtain energy.

Furthermore, the increased OCR was associated with non-mitochondrial respiration, suggesting the existence of extensive ROS signaling caused by increased enzymatic activity of nitric oxide synthases, NADPH oxidase, and other oxygenases[21,22]. Although the glycolytic metabolism of endothelial cells is a protective strategy against oxidative stress[14], insulin can increase ROS production *via* activation of

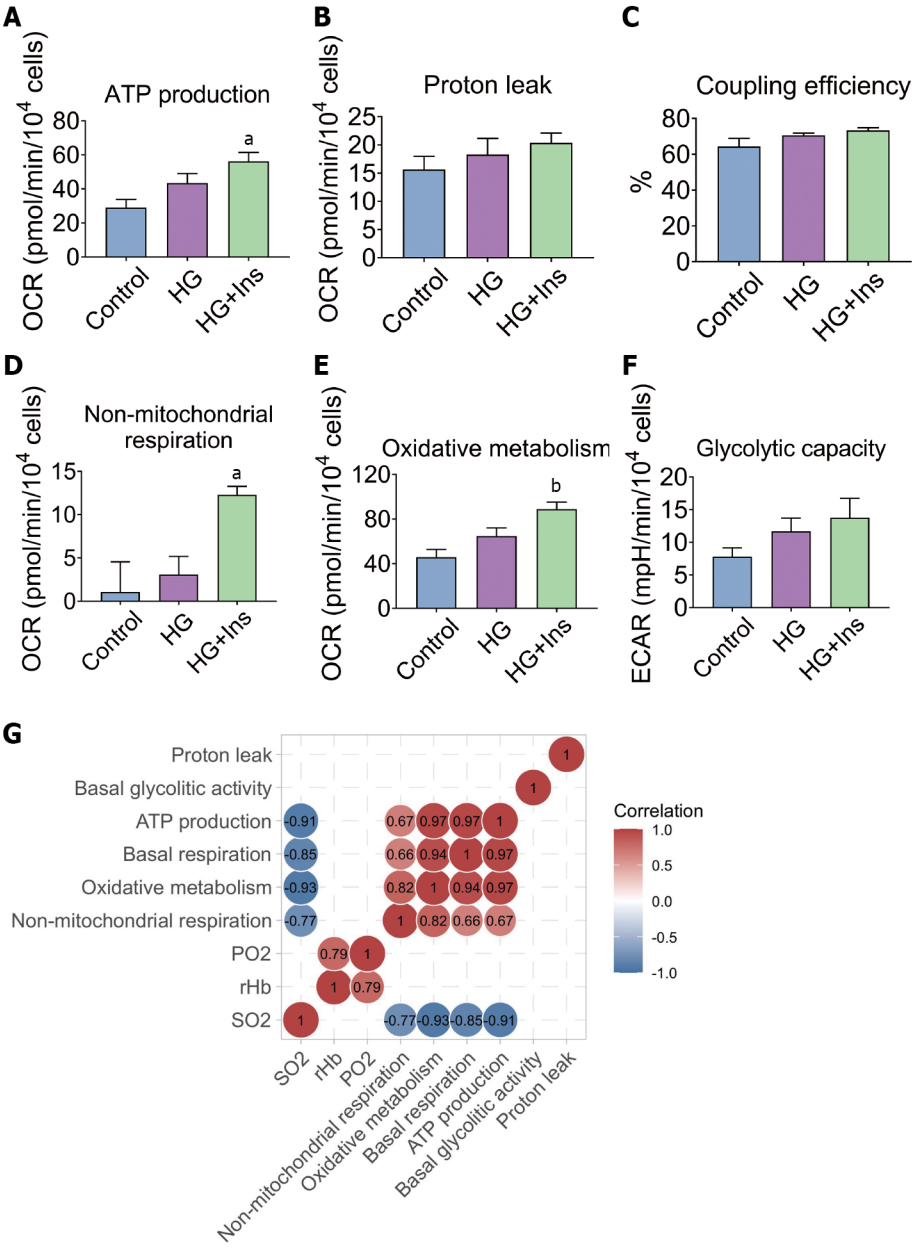

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Figure 5 Metabolic characteristics of the islet microvascular endothelial cells. A-E: Oxygen consumption rates associated with mitochondrial ATP production, proton leak, coupling efficiency, non-mitochondrial respiration and oxidative metabolism; F: Endothelial glycolytic capacity evaluated by extracellular acidification rate; The data are presented as the mean ± SEM, $n = 4$ for each group. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* Control; G: The correlation analysis among pancreatic microcirculatory oxygen profile and microvascular endothelial mitochondrial metabolism. The correlation coefficients (r) in the control, glucotoxicity-exposed, and insulin-treated groups are illustrated as matrix plots. The numbers in the figure represent the correlation coefficient (r) values. SO_2: oxygen saturation; rHb, relative amount of hemoglobin; PO_2: partial oxygen pressure. OCR: Oxygen consumption rate; HG: High glucose; Ins: Insulin.

non-mitochondrial respiration *in vitro*. The excessive ROS levels and increased oxidative stress may lead to mitochondrial dysfunction[23] and endothelial dysfunction[24]. In this energy-demanding process, quiescent endothelial cells divide and migrate to form new vessels[25], and excessive ROS synthesis inhibits angiogenesis by inducing excessive ROS synthesis[26].

Similar to basal respiration, the basal glycolytic activity increases when insulin is present, although no significant difference was noted. An *in vitro* study indicated that insulin, in the context of high glucose, significantly activates oxidative metabolism other than glycolysis in IMECs, although endothelial cells are considered "glycolysis addicted"[27]. The OCR measurements for oxidative metabolism can be divided into three components, including OCR associated with ATP production, proton leak, and non-mitochondrial respiration; the first two indicators together constitute the basal respiration of the mitochondria. Increased ATP production-associated OCR was found in IMECs after insulin treatment, suggesting that mitochondrial energy metabolism participates in the regulatory effects of insulin on microvascular endothelial mitochondrial injury.

The unique role of mitochondria in endothelial cells implies that a cell-regulatory function other than their energy-providing function is dominant[28]. Our previous study indicated that the microvascular blood perfusion of pancreatic islets was significantly decreased in T1DM mice but was partially restored after the administration of insulin[4]. Negative correlations were observed between the microcirculatory oxygen profile and metabolic indexes in the control group. In addition, a relatively low level of mitochondrial metabolism was detected in glycolysis-addicted IMECs, suggesting that glucotoxicity broke the negative correlation due to decreases in microcirculatory perfusion and the oxygen profile.

The current study is the first report on the relationship between pancreatic microcirculatory oxygen profile and microvascular endothelial mitochondrial metabolism. However, there are still several limitations. First, the sample size of mice in each group was limited. Although pancreatic microcirculatory oxygen profile was measured at three random sites of the pancreas in each mouse, large sample size is preferred to ensure the data are representative. Second, in an interdependent functional relationship with β cells, IMECs are involved not only in the delivery of oxygen, but affect adult β cell function and promote β cell proliferation *via* vasoactive substances. However, the phenotypic and functional crosstalk between IMECs and islet β cells are not involved in our study.

CONCLUSION

In conclusion, glucotoxicity deteriorates the integrated pancreatic microcirculatory oxygen profile and bioenergetics, but this deterioration can be reversed by insulin administration.

ARTICLE HIGHLIGHTS

Research background

The pancreatic islet microcirculation adapts its metabolism to cope with limited oxygen availability and nutrient delivery. In diabetes, the balance between oxygen delivery and consumption is impaired. Insulin has been proven to exert complex actions promoting the maintenance of homeostasis of the pancreas under glucotoxicity.

Research motivation

We tried to provide new insight into the relationship between pancreatic microcirculatory oxygen profile and microvascular endothelial mitochondrial metabolism.

Research objectives

To test the hypothesis that insulin administration can improve the integrated pancreatic microcirculatory oxygen profile and bioenergetics.

Research methods

A three-dimensional framework was generated to visualize the pancreatic microcirculatory oxygen profile. The microcirculatory partial oxygen pressure (PO_2), relative hemoglobin (rHb) and hemoglobin oxygen saturation (SO_2) were evaluated in nondiabetic, type 1 diabetes mellitus (T1DM), and insulin-treated mice. An Extracellular Flux Analyzer was used to detect the real-time changes in bioenergetics by measuring the oxygen consumption rate and extracellular acidification rate in islet microvascular endothelial cells (IMECs).

Research results

Insulin administration ameliorated the glucotoxicity-induced decreases in microcirculatory oxygen parameters (PO_2, rHb, and SO_2) and improved the mitochondrial ultrastructural abnormalities in IMECs. Insulin-treated IMECs exhibited significantly greater basal respiration than glucotoxicity-exposed IMECs. An energy map revealed increased energetic metabolism in insulin-treated IMECs, with significantly increased ATP production, non-mitochondrial respiration, and oxidative metabolism. Significant negative correlations were revealed between microcirculatory SO_2 and bioenergetic parameters.

Research conclusions

Glucotoxicity deteriorates the integrated pancreatic microcirculatory oxygen profile and bioenergetics, but this deterioration can be reversed by insulin administration.

Research perspectives

Our understanding of the physiology and pathology of the pancreas islet microvascular endothelial cell in T1DM has been continually enhanced with the advancement of microcirculatory technology in

parallel with rapidly developing bioenergetics that allows us to increase resolution and precision in our investigations.

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FOOTNOTES

Author contributions: Liu MM designed the experiments; Li BW, Li Y, Zhang X, Fu SJ, Wang B, Zhang XY, Liu XT, Wang Q and Li AL performed the experiments; Li BW, Li Y and Liu MM analyzed the data; Li BW and Liu MM wrote the manuscript; Liu MM made critical revisions to the article for important intellectual content; All authors discussed the results and approved the final version of the manuscript.

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REFERENCES

1 **Dybala MP**, Gebien LR, Reyna ME, Yu Y, Hara M. Implications of Integrated Pancreatic Microcirculation: Crosstalk between Endocrine and Exocrine Compartments. *Diabetes* 2020; **69**: 2566-2574 [PMID: 33148810 DOI: 10.2337/db20-0810]

2 **Dybala MP**, Kuznetsov A, Motobu M, Hendren-Santiago BK, Philipson LH, Chervonsky AV, Hara M. Integrated Pancreatic Blood Flow: Bidirectional Microcirculation Between Endocrine and Exocrine Pancreas. *Diabetes* 2020; **69**: 1439-1450 [PMID: 32198213 DOI: 10.2337/db19-1034]

3 **Liu M**, Zhang X, Li A, Wang B, Li B, Liu S, Li H, Xiu R. Insulin treatment restores islet microvascular vasomotion function in diabetic mice. *J Diabetes* 2017; **9**: 958-971 [PMID: 27976498 DOI: 10.1111/1753-0407.12516]

4 **Li Y**, Li B, Wang B, Liu M, Zhang X, Li A, Zhang J, Zhang H, Xiu R. Integrated pancreatic microcirculatory profiles of streptozotocin-induced and insulin-administrated type 1 diabetes mellitus. *Microcirculation* 2021; **28**: e12691 [PMID: 33655585 DOI: 10.1111/micc.12691]

5 **Muratore M**, Santos C, Rorsman P. The vascular architecture of the pancreatic islets: A homage to August Krogh. *Comp Biochem Physiol A Mol Integr Physiol* 2021; **252**: 110846 [PMID: 33197561 DOI: 10.1016/j.cbpa.2020.110846]

6 **Jansson L**, Carlsson PO. Pancreatic Blood Flow with Special Emphasis on Blood Perfusion of the Islets of Langerhans. *Compr Physiol* 2019; **9**: 799-837 [PMID: 30892693 DOI: 10.1002/cphy.c160050]

7 **Wai T**, Langer T. Mitochondrial Dynamics and Metabolic Regulation. *Trends Endocrinol Metab* 2016; **27**: 105-117 [PMID: 26754340 DOI: 10.1016/j.tem.2015.12.001]

8 **Bertero E**, Maack C. Calcium Signaling and Reactive Oxygen Species in Mitochondria. *Circ Res* 2018; **122**: 1460-1478 [PMID: 29748369 DOI: 10.1161/CIRCRESAHA.118.310082]

9 **Li Y**, Song X, Liu M, Wang B, Zhang J, Li A, Zhang H, Xiu R. Multimodal Device and Computer Algorithm-Based Monitoring of Pancreatic Microcirculation Profiles In Vivo. *Pancreas* 2020; **49**: 1075-1082 [PMID: 32769856 DOI: 10.1097/MPA.0000000000001627]

10 **Wang B**, Zhang X, Liu M, Li Y, Zhang J, Li A, Zhang H, Xiu R. Insulin protects against type 1 diabetes mellitus-induced ultrastructural abnormalities of pancreatic islet microcirculation. *Microscopy (Oxf)* 2020; **69**: 381-390 [PMID: 32648910 DOI: 10.1093/jmicro/dfaa036]

11 **Strain WD**, Paldánius PM. Diabetes, cardiovascular disease and the microcirculation. *Cardiovasc Diabetol* 2018; **17**: 57 [PMID: 29669543 DOI: 10.1186/s12933-018-0703-2]

12 **Leone TC**, Kelly DP. Transcriptional control of cardiac fuel metabolism and mitochondrial function. *Cold Spring Harb Symp Quant Biol* 2011; **76**: 175-182 [PMID: 22096028 DOI: 10.1101/sqb.2011.76.011965]

13 **Wong BW**, Marsch E, Treps L, Baes M, Carmeliet P. Endothelial cell metabolism in health and disease: impact of hypoxia. *EMBO J* 2017; **36**: 2187-2203 [PMID: 28637793 DOI: 10.15252/embj.201696150]

14 **Li X**, Sun X, Carmeliet P. Hallmarks of Endothelial Cell Metabolism in Health and Disease. *Cell Metab* 2019; **30**: 414-433 [PMID: 31484054 DOI: 10.1016/j.cmet.2019.08.011]

15 **Yetkin-Arik B**, Vogels IMC, Nowak-Sliwinska P, Weiss A, Houtkooper RH, Van Noorden CJF, Klaassen I, Schlingemann RO. The role of glycolysis and mitochondrial respiration in the formation and functioning of endothelial tip cells during angiogenesis. *Sci Rep* 2019; **9**: 12608 [PMID: 31471554 DOI: 10.1038/s41598-019-48676-2]

16 **Caja S**, Enríquez JA. Mitochondria in endothelial cells: Sensors and integrators of environmental cues. *Redox Biol* 2017; **12**: 821-827 [PMID: 28448943 DOI: 10.1016/j.redox.2017.04.021]

17 **Pinti MV**, Fink GK, Hathaway QA, Durr AJ, Kunovac A, Hollander JM. Mitochondrial dysfunction in type 2 diabetes mellitus: an organ-based analysis. *Am J Physiol Endocrinol Metab* 2019; **316**: E268-E285 [PMID: 30601700 DOI: 10.1152/ajpendo.00314.2018]

18 **Rellick SL**, Hu H, Simpkins JW, Ren X. Evaluation of Bioenergetic Function in Cerebral Vascular Endothelial Cells. *J Vis Exp* 2016 [PMID: 27911398 DOI: 10.3791/54847]

19 **Minami T**, Muramatsu M, Kume T. Organ/Tissue-Specific Vascular Endothelial Cell Heterogeneity in Health and Disease. *Biol Pharm Bull* 2019; **42**: 1609-1619 [PMID: 31582649 DOI: 10.1248/bpb.b19-00531]

20 **De Bock K**, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquière B, Cauwenberghs S, Eelen G, Phng LK, Betz I, Tembuyser B, Brepoels K, Welti J, Geudens I, Segura I, Cruys B, Bifari F, Decimo I, Blanco R, Wyns S, Vangindertael J, Rocha S, Collins RT, Munck S, Daelemans D, Imamura H, Devlieger R, Rider M, Van Veldhoven PP, Schuit F, Bartrons R, Hofkens J, Fraisl P, Telang S, Deberardinis RJ, Schoonjans L, Vinckier S, Chesney J, Gerhardt H, Dewerchin M, Carmeliet P. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* 2013; **154**: 651-663 [PMID: 23911327 DOI: 10.1016/j.cell.2013.06.037]

21 **Nickel A**, Kohlhaas M, Maack C. Mitochondrial reactive oxygen species production and elimination. *J Mol Cell Cardiol* 2014; **73**: 26-33 [PMID: 24657720 DOI: 10.1016/j.yjmcc.2014.03.011]

22 **Lyra-Leite DM**, Andres AM, Cho N, Petersen AP, Ariyasinghe NR, Kim SS, Gottlieb RA, McCain ML. Matrix-guided control of mitochondrial function in cardiac myocytes. *Acta Biomater* 2019; **97**: 281-295 [PMID: 31401347 DOI: 10.1016/j.actbio.2019.08.007]

23 **Vandekeere S**, Dubois C, Kalucka J, Sullivan MR, García-Caballero M, Goveia J, Chen R, Diehl FF, Bar-Lev L, Souffreau J, Pircher A, Kumar S, Vinckier S, Hirabayashi Y, Furuya S, Schoonjans L, Eelen G, Ghesquière B, Keshet E, Li X, Vander Heiden MG, Dewerchin M, Carmeliet P. Serine Synthesis *via* PHGDH Is Essential for Heme Production in Endothelial Cells. *Cell Metab* 2018; **28**: 573-587.e13 [PMID: 30017355 DOI: 10.1016/j.cmet.2018.06.009]

24 **Kalucka J**, Bierhansl L, Conchinha NV, Missiaen R, Elia I, Brüning U, Scheinok S, Treps L, Cantelmo AR, Dubois C, de Zeeuw P, Goveia J, Zecchin A, Taverna F, Morales-Rodriguez F, Brajic A, Conradi LC, Schoors S, Harjes U, Vriens K, Pilz GA, Chen R, Cubbon R, Thienpont B, Cruys B, Wong BW, Ghesquière B, Dewerchin M, De Bock K, Sagaert X, Jessberger S, Jones EAV, Gallez B, Lambrechts D, Mazzone M, Eelen G, Li X, Fendt SM, Carmeliet P. Quiescent Endothelial Cells Upregulate Fatty Acid β-Oxidation for Vasculoprotection *via* Redox Homeostasis. *Cell Metab* 2018; **28**: 881-894.e13 [PMID: 30146488 DOI: 10.1016/j.cmet.2018.07.016]

25 **Carmeliet P**, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature* 2011; **473**: 298-307 [PMID: 21593862 DOI: 10.1038/nature10144]

26 **Zhuo W**, Song X, Zhou H, Luo Y. Arginine deiminase modulates endothelial tip cells *via* excessive synthesis of reactive oxygen species. *Biochem Soc Trans* 2011; **39**: 1376-1381, suppl 2 p following 1382 [PMID: 21936818 DOI: 10.1042/BST0391376]

27 **Pircher A**, Treps L, Bodrug N, Carmeliet P. Endothelial cell metabolism: A novel player in atherosclerosis? *Atherosclerosis* 2016; **253**: 247-257 [PMID: 27594537 DOI: 10.1016/j.atherosclerosis.2016.08.011]

28 **Kadlec AO**, Beyer AM, Ait-Aissa K, Gutterman DD. Mitochondrial signaling in the vascular endothelium: beyond reactive oxygen species. *Basic Res Cardiol* 2016; **111**: 26 [PMID: 26992928 DOI: 10.1007/s00395-016-0546-5]

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ORIGINAL ARTICLE

Retrospective Study

Relationship between age of pregnant women with gestational diabetes mellitus and mode of delivery and neonatal Apgar score

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Abstract

BACKGROUND

Gestational diabetes mellitus (GDM) refers to abnormal glucose tolerance during pregnancy, and it is often accompanied by obvious changes in glucose and lipid metabolism, and associated with adverse pregnancy outcomes. The incidence of fetal distress, polyhydramnios, puerperal infection, premature delivery, and macrosomia in pregnant women with GDM are higher than in those without GDM.

AIM

To analyze the relationship between age of pregnant women with GDM and mode of delivery and neonatal Apgar score.

METHODS

A total of 583 pregnant women with GDM who delivered in the Department of Obstetrics at our hospital between March 2019 and March 2022 were selected. Among them, 377 aged < 35 years were selected as the right age group and 206 aged > 35 years were selected as the older group. The clinical data of the two groups were collected, and the relationship between age of the pregnant women with GDM and mode of delivery, maternal and neonatal outcomes, and neonatal Apgar score were compared. In the older group, 159 women were classed as the adverse outcome group and 47 as the good outcome group according to whether they had adverse maternal and infant outcomes. The related factors of adverse maternal and infant outcomes were analyzed through logistic regression.

RESULTS

The number of women with assisted pregnancy, ≤ 37 wk gestation, ≥ 2 pregnancies, one or more deliveries, and no pre-pregnancy blood glucose screening in the older group were all higher than those in the right age group ($P < 0.05$). The natural delivery rate in the right age group was 40.85%, which was higher than 22.33% in the older group ($P < 0.05$). The cesarean section rate in the older group was 77.67%, which was higher than 59.15% in the right age group ($P < 0.05$). The older group had a higher incidence of polyhydramnios and postpartum hemorrhage, and lower incidence of fetal distress than the right age group had ($P < 0.05$). There was no significant difference in neonatal weight between the two groups ($P > 0.05$). The right age group had higher Apgar scores at 1 and 5 min than the older group had ($P < 0.05$). Significant differences existed between the poor and good outcome groups in age, education level, pregnancy mode, ≤ 37 wk gestation, number of pregnancies, and premature rupture of membranes ($P < 0.05$). Logistic regression showed that age, education level and premature rupture of membranes were all risk factors affecting the adverse outcomes of mothers and infants ($P < 0.05$).

CONCLUSION

Delivery mode and Apgar score of pregnant women with GDM are related to age. Older age increases the adverse outcome of mothers and infants.

Key Words: Gestational diabetes mellitus; Age; Mode of delivery; Neonatal Apgar score

Core Tip: This study analyzed the relationship between the age of pregnant women with gestational diabetes mellitus (GDM) and mode of delivery and neonatal Apgar score. Pregnant women with GDM were divided into right age and older groups. Compared with the older group, the natural delivery rate in the right age group was higher, but the cesarean section rate was lower. Moreover, age, education level and premature rupture of membranes were associated with the adverse outcomes of mothers and infants. Age was related to the delivery mode and Apgar score of pregnant women with GDM.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the specific diseases of pregnant women. It refers to abnormal glucose tolerance in different degrees during pregnancy, often accompanied by obvious changes in glucose and lipid metabolism, and is closely related to adverse pregnancy outcomes. The incidence rate of GDM is increasing annually in China. GDM results in a high-risk pregnancy, which can induce complications, such as abortion, premature delivery, amniotic fluid and infection[1,2]. The incidence of fetal distress, polyhydramnios, puerperal infection, premature delivery, and macrosomia in pregnant women with GDM are higher than in those without GDM[3,4]. Pregnancy at the right age is the key to reduce the risk of adverse outcomes. With the implementation of China's three-child policy, the number of pregnant women with advanced maternal age is gradually increasing, and the incidence of pregnancy complications is significantly higher than that in right-age pregnant women. In recent years, with the rapid development of medical technology, significant progress has been made in the treatment of birth defects and premature infants. However, there are no effective measures to avoid the adverse outcomes of older-age pregnancies, especially those with GDM. Previous studies have shown that body mass index (BMI) may have an impact on maternal and neonatal outcomes in pregnant women with GDM[5,6]. However, pregnancy is still a risk factor for adverse maternal and neonatal outcomes and is affected by many factors. The clinical data of 583 pregnant women with GDM who delivered in the Department of Obstetrics at our hospital between March 2019 and March 2022 were retrospectively analyzed. The delivery mode and neonatal Apgar score of pregnant women with GDM at different ages were compared, to improve the pregnancy outcome of the older pregnant women, and provide a reference for ensuring the effect of eugenics and prenatal care.

MATERIALS AND METHODS

General data

The clinical data of 583 pregnant women with GDM who delivered in the Department of Obstetrics at our hospital between March 2019 and March 2022 were retrospectively analyzed. This study was approved by the Ethics Committee of our hospital. Inclusion criteria were: (1) All women conformed to the clinical diagnostic criteria for GDM[7]; (2) regular pregnancy examination; (3) successful delivery; (4) no hereditary diseases of coagulation system; (5) complete clinical medical records; and (6) pregnant women and family members gave informed consent to participate in this study.

Exclusion criteria were: (1) Women were diagnosed with diabetes mellitus or impaired glucose regulation before pregnancy; (2) women with other pregnancy-related diseases; (3) heart, liver or kidney dysfunction; (4) hematological diseases; (5) other diseases that may affect blood glucose; and (6) mental illness or retardation. Among the selected pregnant woman, 377 aged < 35 years were selected as the right age group and 206 aged > 35 years were selected as the older group. The data flow chart is shown in Figure 1.

Clinical data selection

The clinical data of the two groups were collected, including: age; pregnancy mode; educational level; BMI; fasting blood glucose; gestational weeks of delivery; number of pregnancies; number of deliveries; pre-pregnancy blood glucose screening; maternal and infant outcomes (preterm birth, polyhydramnios, oligohydramnios, fetal distress, macrosomia, umbilical cord around the neck, neonatal death events, neonatal hospitalization, neonatal aspiration pneumonia, neonatal hypoglycemia, neonatal jaundice, and postpartum hemorrhage); and Apgar scores at 1 and 5 min after birth. The clinical data of pregnant women in the two groups were retrospectively analyzed. In the older group, 159 women were classed as the adverse outcome group and 47 as the good outcome group according to whether they had adverse maternal and infant outcomes.

Diagnostic criteria

GDM was diagnosed by the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria. The IADPSG recommends testing to be routinely carried out between 24 and 28 wk of gestation or at the first prenatal visit in high-risk women. Based on the results of a 75-g, 2-h oral glucose tolerance test, a woman was diagnosed with GDM when one or more of her plasma glucose concentrations were equivalent to or exceeded the following levels: fasting, 92 mg/dL; 1 h, 180 mg/dL; or 2 h, 153 mg/dL.

Statistical analysis

The data in this study were analyzed using SPSS 21.0. The measurement data were expressed as mean ± SD, and were compared using the independent sample *t* test between two groups. The enumeration data were expressed as *n* (%), and were compared using the χ^2 test between two groups. Factors related to adverse maternal and infant outcomes in older pregnant women were analyzed using logistic regression analysis. $P < 0.05$ was regarded as statistically significant.

RESULTS

Comparison of general data of pregnant women with GDM in two groups

The number of women with assisted pregnancy, ≤ 37 wk gestation, ≥ 2 pregnancies, one or more deliveries, and no pre-pregnancy blood glucose screening in the older group were all higher than those in the right age group ($P < 0.05$) (Table 1).

Comparison of delivery modes between the two groups

The right age pregnant women had a higher natural delivery rate of 40.85% compared with 22.5% in the older group ($P < 0.05$). The older group had a higher cesarean section rate of 77.67% compared with 59.15% in the right age group ($P < 0.05$) (Table 2).

Comparison of adverse outcomes between two groups

The older group had a higher incidence of polyhydramnios and postpartum hemorrhage, and lower incidence of fetal distress than the right age group had ($P < 0.05$) (Table 3).

Comparison of Apgar score between two groups

No significant difference existed in neonatal weight between the two groups ($P > 0.05$). The right age group had higher Apgar scores at 1 and 5 min after birth than the older group had ($P < 0.05$) (Table 4).

Table 1 Comparison of general data of pregnant women with gestational diabetes mellitus in two groups

General data		Right age group (n = 377)	Older group (n = 206)	t/χ^2	P
Pregnancy mode	Natural pregnancy	343 (90.98)	170 (82.52)	9.018	0.003
	Assisted pregnancy	34 (9.02)	36 (17.48)		
Education level	Primary school and below	4 (1.06)	5 (2.43)	5.054	0.080
	Junior high school	80 (21.22)	57 (27.67)		
	College degree or above	293 (77.72)	144 (69.90)		
BMI before pregnancy (kg/m^2)		22.30 ± 3.77	22.80 ± 3.75	1.534	0.126
Gestational weight gain (kg)		10.83 ± 15.21	9.20 ± 16.15	1.210	0.227
FBG (mmol/L)		4.93 ± 1.14	4.89 ± 0.69	0.460	0.646
≤ 37 wk gestation		110 (29.18)	84 (40.78)	8.072	0.004
No. of Pregnancies	1	134 (35.54)	24 (11.65)	38.493	< 0.001
	≥ 2	243 (64.46)	182 (88.35)		
Delivery times	0	226 (59.95)	59 (28.64)	52.441	< 0.001
	1	129 (34.22)	123 (59.71)		
	≥ 2	22 (5.84)	24 (11.65)		
Pre-pregnancy blood glucose screening	Yes	159 (42.18)	67 (32.52)	5.227	0.022
	No	218 (57.82)	139 (67.48)		

BMI: Body mass index; FBG: Fasting blood glucose.

Table 2 Comparison of delivery modes between the two groups

Groups	Cases	Natural delivery	Cesarean section rate
Right age group	377	154 (40.85)	223 (59.15)
Older group	206	46 (22.33)	160 (77.67)
χ^2		20.271	
P		< 0.001	

Analysis of related factors of adverse maternal and infant outcomes in older pregnant women

Significant differences existed between the poor (n = 159) and good (n = 47) outcome groups for age, education level, pregnancy mode, ≤ 37 wk gestation weeks, number of pregnancies, and premature rupture of membranes (P < 0.05) (Table 5).

Logistic regression analysis of risk factors for maternal and infant adverse outcomes in older pregnant women with GDM

The interference between the various indicators was controlled and the correlation between these indicators and maternal and infant adverse outcomes in older women with GDM was analyzed by logistic regression analysis. The analysis was conducted using significant factors in Table 5 as independent variables and adverse maternal and infant outcomes as the dependent variable. The regression model was established by selecting the indexes such as age, education level, pregnancy mode, ≤ 37 wk gestation, number of pregnancies, and premature rupture of membranes. Logistic regression showed that age, education level and premature rupture of membranes were risk factors for maternal and infant adverse outcomes in older women with GDM (P < 0.05) (Table 6 and nomogram analysis in Figure 2).

DISCUSSION

GDM is a special type of diabetes with a morbidity of 17.5%-18.9%, and the morbidity increases with

Table 3 Comparison of adverse outcomes between two groups

Adverse outcomes	Right age group (*n* = 377)	Older group (*n* = 206)	t/χ^2	*P*
Preterm birth	40 (10.61)	26 (12.62)	0.37	0.464
Polyhydramnios	5 (1.32)	8 (3.88)	3.996	0.046
Oligohydramnios	32 (8.49)	10 (4.85)	2.631	0.105
Fetal distress	40 (10.61)	10 (4.85)	5.628	0.018
Macrosomia	8 (2.12)	2 (0.97)	1.047	0.306
Umbilical cord around the neck	125 (33.16)	59 (28.64)	1.258	0.262
Neonatal death events	3 (0.80)	1 (0.49)	0.188	0.664
Neonatal hospitalization	4 (0.27)	1 (0.49)	0.519	0.471
Neonatal aspiration pneumonia	3 (0.80)	2 (0.97)	0.048	0.827
Neonatal Hypoglycemia	4 (0.27)	2 (0.97)	0.011	0.918
neonatal jaundice	5 (1.32)	2 (0.97)	0.142	0.706
Postpartum hemorrhage (mL)	318.62 ± 97.02	362.20 ± 175.92	3.861	0.001

Table 4 Comparison of Apgar score between the two groups

Groups	Cases	Neonatal weight (g)	1 min Apgar score	5 min Apgar score
Right age group	377	3107.66 ± 467.26	9.69 ± 0.06	9.93 ± 0.05
Older group	206	3102.07 ± 508.40	9.67 ± 0.08	9.89 ± 0.04
χ^2		0.134	3.408	9.882
P		0.894	0.001	< 0.001

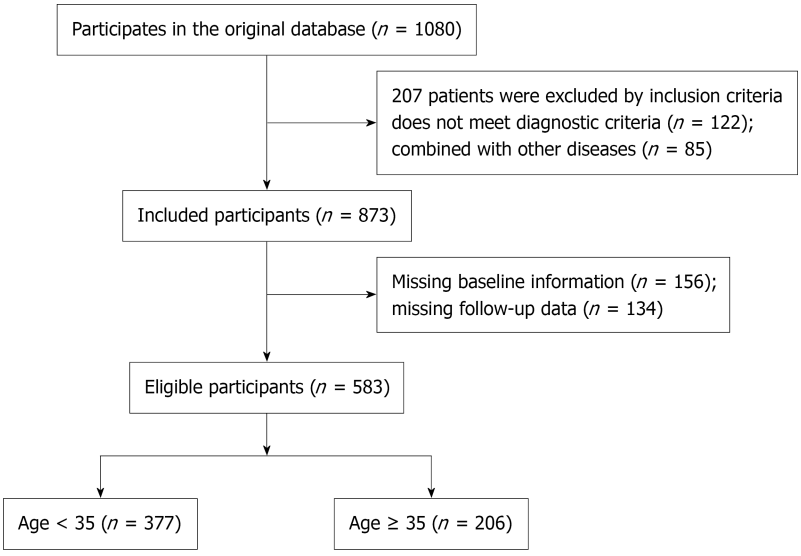

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Figure 1 Flow chart of general data selection.

age[8]. GDM increases the morbidity of pregnancy-related complications, which could lead to adverse pregnancy outcomes such as premature delivery, cesarean section, macrosomia and premature rupture of membranes, thus attracting the attention of the majority of medical staff and pregnant women[9,10]. Some scholars have found that gestational age > 35 years, pre-pregnancy BMI, and family history of diabetes are all risk factors for GDM, which increasing the incidence of adverse pregnancy outcomes such as premature delivery, macrosomia and fetal distress[11,12]. Previous studies have mainly focused on the high-risk factors of GDM[13-15], and have confirmed that advanced age is a risk factor for GDM.

Table 5 Analysis of related factors of adverse maternal and infant outcomes in older pregnant women

Factors		Poor outcome group (*n* = 112)	Good outcome group (*n* = 94)	t/χ^2	*P*
Age		38.75 ± 1.26	37.26 ± 1.78	7.011	< 0.001
Education level	Primary school and below	0 (0.00)	5 (5.32)	6.257	0.044
	Junior high school	33 (29.46)	24 (25.53)		
	College degree or above	79 (70.54)	65 (69.15)		
Pregnancy mode	Natural pregnancy	87 (77.68)	83 (88.30)	3.996	0.046
	Assisted reproduction	25 (22.32)	11 (11.70)		
≤ 37 wk gestation		53 (47.32)	31 (32.98)	4.354	0.037
No. of pregnancies	1	18 (16.07)	6 (6.38)	4.661	0.031
	≥ 2	94 (83.93)	88 (93.62)		
No. of deliveries	0	36 (32.14)	23 (24.47)	1.543	0.462
	1	63 (56.25)	60 (63.83)		
	≥ 2	13 (11.61)	11 (11.70)		
Mode of delivery	Natural labor	25 (22.32)	21 (22.34)	0.000	0.997
	Cesarean section	87 (77.68)	73 (77.66)		
Premature rupture of membranes		36 (32.14)	0 (0.00)	36.613	< 0.001

Table 6 Logistic regression analysis of risk factors for maternal and infant adverse outcomes in elderly pregnant women with gestational diabetes mellitus

Risk factors	B	SE	Wald χ^2	*P*	OR	95%CI
Age	0.485	0.074	15.090	0.005	1.254	1.002-4.056
Education level	0.650	0.112	20.482	0.019	1.234	1.051-5.573
Pregnancy mode	0.253	0.145	2. 774	0.045	1.254	0.976-1.780
≤ 37 wk gestation	0.504	0.256	3.157	0.086	1.643	0.949-2.954
No. of pregnancies	0.784	0.165	5.48	0.097	1.262	0.758-1.985
Premature rupture of membranes	0.864	0.142	16.751	0.011	1.318	1.185-9.254

SEM: Standard error of mean; OR: Odd ratio; CI: Confidence interval.

However, there has been less research on older women with GDM. In this study, we retrospectively analyzed the clinical data of older and right-age pregnant women with GDM. The general situation, delivery mode, and maternal and neonatal outcomes were compared, and the delivery characteristics of women with GDM at different ages were discussed, aiming to improve pregnancy outcome in the older age group and providing a reference for ensuring the effect of eugenics in China.

The number of women with assisted pregnancy, ≤ 37 wk gestation, ≥ 2 pregnancies, one or more deliveries, and no pre-pregnancy blood glucose screening in the older group were all higher than those in the right-age group. The ovarian function of older women decreased significantly, and the ovarian reserve and egg quality decreased gradually, resulting in a decline in successful pregnancy rate. Assisted reproductive technology helps a large number of infertile older women conceive successfully, but the complications during pregnancy are higher than those of pregnant women who conceive naturally. With the implementation of the three-child policy in China, the number of older pregnant women is increasing gradually. Older women have a higher incidence of pregnancy complications, such as GDM, and higher risk of maternal and infant deaths than pregnant women at the right age have. Therefore, we should attach importance to the healthcare of older women during pregnancy and in the perinatal period. The results of the present study showed that the older group had a higher cesarean section rate of 77.67%, compared with 59.15% in the right-age group. The right age group had higher Apgar scores at 1 and 5 min after birth compared with the older group. In recent years, the rate of cesarean section has increased due to incorrect fetal position or prevention of fetal distress. There are many pregnancy complications in older women, uterine contraction is weaker, and the total labor

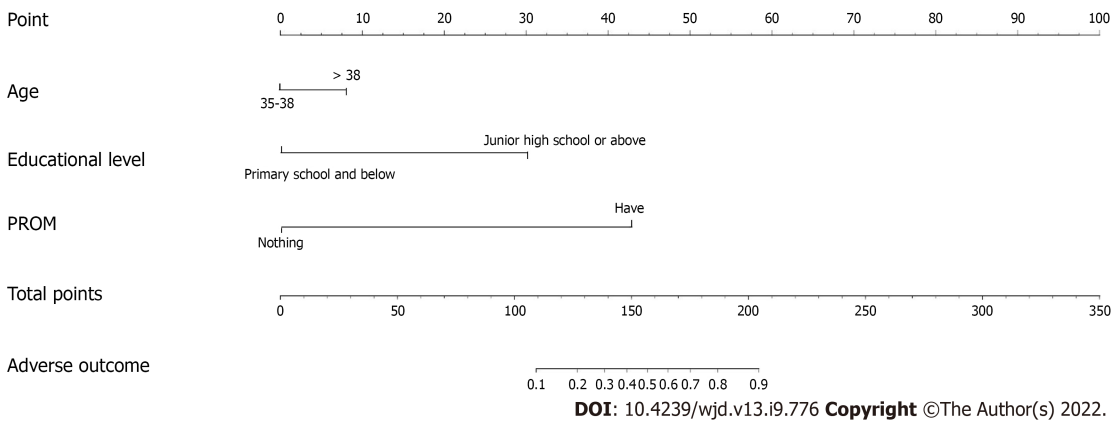

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Figure 2 Nomogram analysis of risk factors for adverse maternal and infant outcomes in older pregnant women. PROM: Premature rupture of membrane.

process is prolonged. Therefore, natural delivery can increase maternal and neonatal risks. This is one of the reasons why older women choose cesarean section, but this increases the risk of maternal and neonatal infection and thromboembolism[8,16-18]. With increasing age, pregnant women are prone to obesity, and various bodily functions gradually decrease. In older pregnant women with GDM, the incidence of fetal macrosomia is high. We showed that older women had a higher incidence of polyhydramnios and postpartum hemorrhage than the right-age women had, which confirms that GDM is closely related to maternal and infant adverse outcomes. According to previous studies, older age pregnancy has a higher risk of GDM[19-21]. Older age can also increase the probability of gestational hypertension, perinatal complications and other diseases. The present study found that the older group had a lower incidence of fetal distress than the right-age group had, which may be related to the high rate of cesarean section in the older group. The incidence of fetal distress was reduced due to the high proportion of women with ≤ 37 wk gestation in the older group.

To control the interference between the various indicators and analyze the correlation between related indicators and maternal and infant adverse outcomes in older women with GDM, logistic regression analysis was conducted. Logistic regression showed that age, education level and premature rupture of membranes were risk factors for maternal and infant adverse outcomes in older women with GDM. Pregnant women aged > 35 years old are more likely to have pregnancy complications during pregnancy or delivery. Laura estimated the risk of adverse outcome over a pregnancy cycle of 3-24 mo based on the maternal age at the initial birth (20-34 years and ≥ 35 years)[20]. The risk of maternal mortality or serious morbidity at 6 mo of pregnancy was increased compared to the 18-mo pregnancy cycle of women aged ≥ 35 years. Women aged 20-34 years had an increased risk of spontaneous preterm birth within 6 mo of pregnancy. In the present study, with the increase in academic qualifications, the proportion of maternal and infant adverse outcomes in older pregnant women with GDM increased, and there was a significant difference compared with the good outcome group. Education level is a risk factor for maternal and infant adverse outcomes in older women with GDM. The reason may be that the higher the educational background is, the later the childbearing age is, which affects fertility and reproductive quality. Premature rupture of membranes induces serious adverse effects in both the mother and fetus. In premature rupture of membranes, the reproductive tract loses its protective barrier, and the amniotic fluid gradually decreases, which affects the blood circulation of the placenta and increases proneness to adverse outcomes such as fetal distress[22,23]. Obstetric medical staff should focus on monitoring pregnant women with high-risk factors of premature rupture of membranes and implement timely intervention measures.

The limitation of this study was that the subjects were from a single institution, which limits the extrapolation of research results. More eligible samples will be included in future studies and more disease-related data will be analyzed, to enhance the reliability and validity of the results and provide a basis for treatment.

CONCLUSION

In summary, the delivery mode and neonatal Apgar score are related to the age of pregnant women with GDM and advanced age increases the adverse outcomes in mothers and infants. Therefore, to improve the pregnancy outcome and reduce the incidence of complications in pregnant women with GDM, it is suggested that pregnant women with a family planning plan should have pre-pregnancy eugenics health examination and pregnancy health care.

ARTICLE HIGHLIGHTS

Research background

Gestational diabetes mellitus (GDM) is one of the serious pregnancy complications, which severely threatens the health of pregnant women and newborns. In recent years, with the increased childbearing age and proportion of overweight people, the incidence of GDM has an upward trend. The detrimental effect of GDM on the prognosis has been recognized, which can increase the incidence of dystocia, cesarean section and macrosomia, and 17%-63% of pregnant women and infants will develop type 2 diabetes in the long term.

Research motivation

We analyzed the risk factors affecting the adverse outcomes of mothers and infants, we also put forward targeted prevention and control measures to provide reference for formulating GDM early prevention and intervention policies.

Research objectives

To explore the relationship between the age of GDM pregnant women and the delivery mode and neonatal Apgar score, so as to provide a theoretical basis for reducing the incidence of adverse pregnancy outcomes.

Research methods

We used the latest diagnostic criteria of GDM to investigate pregnant women who met the inclusion criteria, and collected their general conditions before and during pregnancy and related clinical data. The women were divided into right age group and older group according to whether they were older than 35 years old. Logistic regression analysis was used to analyze the related risk factors affecting the delivery outcome of GDM pregnant women.

Research results

The older group had a higher cesarean section rate, higher incidence of polyhydramnios and postpartum hemorrhage, and lower incidence of fetal distress than the right age group. The right age group had higher Apgar scores at 1 and 5 min after birth than the older group. Moreover, age, education level and premature rupture of membranes were risk factors for adverse pregnant outcomes in older GDM women. Our results showed that gestational age greater than 35 years old will increase the incidence of gestational diabetes and adverse pregnancy outcomes, but it needs to be confirmed by large samples and studies involving patients in multiple research centers.

Research conclusions

The age of pregnant women with GDM affects the delivery mode and neonatal Apgar score, and advanced age increases the adverse pregnancy outcomes. Therefore, it is suggested that pregnant women should have pre-pregnancy eugenics health examination and pregnancy health care.

Research perspectives

In the future research, we want to explore the effective means of screening GDM in pregnancy physical examination, as well as the means of early intervention and treatment for pregnant women with high-risk factors of GDM.

FOOTNOTES

Author contributions: Gao L and Chen CR contributed equally to this work; Gao L and Chen CR drafted the manuscript; Yang Y provision patients; Gao L, Wang F, Ji Q collected the data; Chen CR and Liu HW analyzed and interpreted the data; Liu HW contributed to conception and design; Chen KN contributed to administrative support.

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Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

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REFERENCES

1 **Dall TM**, Yang W, Gillespie K, Mocarski M, Byrne E, Cintina I, Beronja K, Semilla AP, Iacobucci W, Hogan PF. The Economic Burden of Elevated Blood Glucose Levels in 2017: Diagnosed and Undiagnosed Diabetes, Gestational Diabetes Mellitus, and Prediabetes. *Diabetes Care* 2019; **42**: 1661-1668 [PMID: 30940641 DOI: 10.2337/dc18-1226]
2 **Drehmer M**, Silveira L, Bracco P, Schmidt MI. Postpartum weight retention and pregnancy weight gain in women with Gestational Diabetes Mellitus (GDM). *Int J Epidemiol* 2021; **50**: 1311 [DOI: 10.1093/ije/dyab168.170]
3 **Li H**, Song L, Shen L, Liu B, Zheng X, Zhang L, Wang Y, Cao Z, Xu S. Height and Risk of Gestational Diabetes Mellitus: Results from the Healthy Baby Cohort Study. *J Diabetes Res* 2018; **2018**: 4679245 [PMID: 30159334 DOI: 10.1155/2018/4679245]
4 **Remneva OV**, Rozhkova OV, Cherkasova TM, Korenovskiy YV, Gurevich NL. Clinical, metabolic and neurological disorders in full-term newborns from mothers with gestational diabetes mellitus. *Rossiyskiy Vestnik Perinatologii i Pediatrii (Russian Bulletin of Perinatology and Pediatrics)* 2021; **66**: 46-51 [DOI: 10.21508/1027-4065-2021-66-3-46-51]
5 **Corrado F**, Pintaudi B. Diagnosis of Gestational Diabetes Mellitus: Italian Perspectives on Risk Factor-Based Screening. *Nutrition and Diet in Maternal Diabetes* 2018; 87-97 [DOI: 10.1007/978-3-319-56440-1_9]
6 **KhushBakht D**, Mazhar S, Bhalli A, Rashid A, Khan K, Jahanzaib U. Correlation Between Neck Circumference and Gestational Diabetes Mellitus and Associated Risk Factors During Pregnancy. *Cureus* 2018; **10**: e2699 [PMID: 30062073 DOI: 10.7759/cureus.2699]
7 **Harreiter J**, Simmons D, Desoye G, Corcoy R, Adelantado JM, Devlieger R, van Assche A, Galjaard S, Damm P, Mathiesen ER, Jensen DM, Andersen LL, Dunne F, Lapolla A, Dalfra MG, Bertolotto A, Mantaj U, Wender-Ozegowska E, Zawiejska A, Hill D, Jelsma JG, Snoek FJ, Worda C, Bancher-Todesca D, van Poppel MN, Kautzky-Willer A; DALI Core Investigator group. IADPSG and WHO 2013 Gestational Diabetes Mellitus Criteria Identify Obese Women With Marked Insulin Resistance in Early Pregnancy. *Diabetes Care* 2016; **39**: e90-e92 [PMID: 27208336 DOI: 10.2337/dc16-0200]
8 **Karasneh RA**, Migdady FH, Alzoubi KH, Al-Azzam SI, Khader YS, Nusair MB. Trends in maternal characteristics, and maternal and neonatal outcomes of women with gestational diabetes: A study from Jordan. *Ann Med Surg (Lond)* 2021; **67**: 102469 [PMID: 34178318 DOI: 10.1016/j.amsu.2021.102469]
9 **Yoles I**, Sheiner E, Wainstock T. First pregnancy risk factors and future gestational diabetes mellitus. *Arch Gynecol Obstet* 2021; **304**: 929-934 [PMID: 33811260 DOI: 10.1007/s00404-021-06024-8]
10 **Bayoumi MAA**, Masri RM, Matani NYS, Hendaus MA, Masri MM, Chandra P, Langtree LJ, D'Souza S, Olayiwola NO, Shahbal S, Elmalik EE, Bakry MS, Gad AI, Agarwal R. Maternal and neonatal outcomes in mothers with diabetes mellitus in qatari population. *BMC Pregnancy Childbirth* 2021; **21**: 651 [PMID: 34560839 DOI: 10.1186/s12884-021-04124-6]
11 **Hiersch L**, Berger H, Okby R, Ray JG, Geary M, McDonald SD, Murray-Davis B, Riddell C, Halperin I, Hasan H, Barrett J, Melamed N; DOH-NET (Diabetes, Obesity and Hypertension in Pregnancy Research Network); SOON (Southern Ontario Obstetrical Network) Investigators. Gestational diabetes mellitus is associated with adverse outcomes in twin pregnancies. *Am J Obstet Gynecol* 2019; **220**: 102.e1-102.e8 [PMID: 30595142 DOI: 10.1016/j.ajog.2018.10.027]
12 **Shin D**, Lee KW. High pre-pregnancy BMI with a history of gestational diabetes mellitus is associated with an increased risk of type 2 diabetes in Korean women. *PLoS One* 2021; **16**: e0252442 [PMID: 34086709 DOI: 10.1371/journal.pone.0252442]
13 **Nath J**. Gestational diabetes mellitus and perinatal complications - A clinical study. *Indian J Obstet Gynaecol* 2017; **4**: 120-122 [DOI: 10.18231/2394-2754.2017.0027]
14 **Ushanova FO**, Lobanova KG, Perekhodov SN. Gestational diabetes mellitus: peculiarities of course and pregnancy outcomes in real clinical practice. *Meditsinskiy sovet = Medical Council* 2021; 184-191 [DOI: 10.21518/2079-701X-2021-7-184-191]
15 **Balagopalan N**, Pathak R, Islam F, Nigam A, Agarwal S. Diagnostic accuracy of diabetes in pregnancy study group of india criteria for the screening of gestational diabetes mellitus in primary care setting. *Indian J Commun and Fam Med* 2021; **7**: 25 [DOI: 10.4103/IJCFM.IJCFM_9_20]
16 **Bianchi C**, de Gennaro G, Brocchi A, Minaldi E, Del Prato S, Bertolotto A. Risk factors associated with postpartum

impaired glucose regulation in women with previous gestational diabetes. *J Diabetes Complications* 2021; **35**: 107854 [PMID: 33468397 DOI: 10.1016/j.jdiacomp.2021.107854]

17 **Getaneh T**, Asres A, Hiyaru T, Lake S. Adverse perinatal outcomes and its associated factors among adult and advanced maternal age pregnancy in Northwest Ethiopia. *Sci Rep* 2021; **11**: 14072 [PMID: 34234283 DOI: 10.1038/s41598-021-93613-x]

18 **Biagioni EM**, May LE, Broskey NT. The impact of advanced maternal age on pregnancy and offspring health: A mechanistic role for placental angiogenic growth mediators. *Placenta* 2021; **106**: 15-21 [PMID: 33601220 DOI: 10.1016/j.placenta.2021.01.024]

19 **Frick AP**. Advanced maternal age and adverse pregnancy outcomes. *Best Pract Res Clin Obstet Gynaecol* 2021; **70**: 92-100 [PMID: 32741623 DOI: 10.1016/j.bpobgyn.2020.07.005]

20 **Schummers L**, Hutcheon JA, Hernandez-Diaz S, Williams PL, Hacker MR, VanderWeele TJ, Norman WV. Association of Short Interpregnancy Interval With Pregnancy Outcomes According to Maternal Age. *JAMA Intern Med* 2018; **178**: 1661-1670 [PMID: 30383085 DOI: 10.1001/jamainternmed.2018.4696]

21 **Yeim KO**, Snmez E. Impact of maternal age on performance of the progeny in Galleria mellonella (L. 1758) (Lepidoptera: Pyralidae). *Turkish J Ent* 2021; **45**: 33-38 [DOI: 10.16970/ENTOTED.682212]

22 **Ruth H**, Janine Z, Jana P, Yvonne H, Ekkehard S. Placenta inflammation, vaginal microbiome and early-onset neonatal sepsis (eons) after preterm premature rupture of membranes (pprom). *Placenta* 2021; **112**: e66 [DOI: 10.1016/j.placenta.2021.07.212]

23 **Puhl A**, Weiss C, Schneid A, Zahn E, Kraft K, Pretscher J, Faschingbauer F, Beckmann MW, Kehl S. [Does Induction of Labor for Preterm Premature Rupture of Membranes at 34 Weeks of Gestation Increase the Risk for Cesarean Section? *Z Geburtshilfe Neonatol* 2020; **224**: 269-274 [PMID: 32120446 DOI: 10.1055/a-1110-1132]

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Abstract

BACKGROUND

Insulin resistance is a risk factor for metabolic syndromes and is associated with a wide variety of metabolic illnesses, including obesity, type 2 diabetes, and cardiovascular disease.

AIM

To investigate and map global insulin resistance studies.

METHODS

A bibliometric methodology was applied to the literature retrieved from the Scopus database and *Reference Citation Analysis* (https://www.referencecitationanalysis.com) by using a validated search strategy. The study period was limited from 2002 to 2021. Bibliometric indicators and mapping were presented.

RESULTS

A total of 26808 articles on the topic of insulin resistance were included in the Scopus database. The articles included research articles (n = 21918; 81.76%), review articles (n = 2641; 9.85%), and letters (n = 653; 2.44%). During the study period, 136 countries contributed to the research on insulin resistance. The highest number of articles was from the United States (n = 7360; 27.45%), followed by China (n = 3713; 13.85%), Japan (n = 1730, 6.45%), Italy (n = 1545; 5.54%), and the United Kingdom (n = 1484; 5.54%). The retrieved articles identified two main research themes: "inflammatory mechanisms in the regulation of insulin resistance" and "mechanisms linking obesity to insulin resistance".

CONCLUSION

Our data show that insulin resistance has steadily gained interest from researchers, as evidenced by the number of citations and yearly publications. Publications have grown significantly in the last decade, while low-income countries with greater burdens continue to produce fewer publications in this field. This approach might assist researchers in choosing new research areas and recognizing research hotspots and frontiers. In the future, perhaps high-quality clinical evidence will be acquired.

Key Words: Insulin resistance; Research hotspots; Scopus; VOSviewer; Bibliometric

Core Tip: Several bibliometric studies have been conducted in the field of diabetes research. However, no bibliometric study has been conducted on insulin resistance research. Therefore, the current study aims to investigate and map global research on insulin resistance. The retrieved articles identified two main research themes: "inflammatory mechanisms in the regulation of insulin resistance" and "mechanisms linking obesity to insulin resistance". This approach might assist researchers in choosing new research areas and recognizing research hotspots and frontiers. In the future, perhaps high-quality clinical evidence will be acquired.

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INTRODUCTION

During the last two decades, the global prevalence of diabetes has increased dramatically. Diabetes is increasing worldwide, both in terms of prevalence and the number of affected[1]. For more than half a century, insulin resistance and type 2 diabetes have been associated. Insulin resistance is not only a powerful predictor of future type 2 diabetes development but is also a therapeutic target in the presence of hyperglycemia[2]. Insulin resistance is defined as a reduced physiological response to insulin stimulation of target tissues, especially adipose tissue, liver, and muscle. Insulin resistance limits glucose disposal, leading to a compensatory increase in beta cell insulin synthesis and hyperinsulinemia

[3]. More than 30 years ago, hyperinsulinemia and insulin resistance were hypothesized to be key contributors to hypertension, hyperglycemia, dyslipidemia, hyperuricemia, visceral adiposity, elevated inflammatory markers, prothrombic state, and endothelial dysfunction related to obesity and the metabolic syndrome[4].

Several bibliometric studies have been conducted in diabetes research[5-9] or in depression and insulin research[10]. However, no bibliometric study has been conducted on insulin resistance research. As a scientific evaluation approach, bibliometrics can assess the research impact of organizations and individuals[11]. Similarly, bibliometrics provide evidence to promote the formation of future research hotspots[12,13]. As a result, this research aims to examine the scientific development in insulin resistance thoroughly. Therefore, this bibliometric analysis was designed to examine the research trend related to insulin resistance and identify future research hotspots. Furthermore, the study offers some important information by providing references and ideas for future studies on insulin resistance pathophysiology and clinical applications.

MATERIALS AND METHODS

Data acquisition

The documents in the current study were obtained and downloaded from the Scopus database on January 29, 2022 to prevent bias caused by the database's daily updates. With more than 36000 titles from around 11678 publishers, of which 34346 were peer-reviewed journals, Scopus is one of the most extensive and authoritative databases for collecting academic information[14,15]. Unfortunately, only one database may be utilized in bibliometric analyses because data from many databases cannot be integrated and analyzed. On the other hand, systematic reviews use multiple databases to retrieve a large number of documents for further analysis[16]. Furthermore, only one database was chosen on the topic and objective coverage, and past research has shown that Web of Science and PubMed are included in the Scopus database. Based on previous studies and findings, it was recommended to use Scopus (Elsevier database) because it was the most comprehensive database on the subject, offering all the data needed for quantitative analysis[17,18].

Search strategy

Keywords used in the Scopus engine to achieve the aim of this study were chosen from previous systematic reviews and meta-analyses on insulin resistance[19-21]. "Insulin resistance" or "insulin sensitivity" was used as a search expression in the title search in the Scopus database over the last two decades (January 2002 to December 2021). This study used the keywords "insulin resistance" or "insulin sensitivity" because we are more interested in these terms than related terminology. Therefore, keywords were used instead of a title/abstract search in the title search. Consequently, the search for the title will provide the fewest false positive documents, making it a trustworthy strategy[22-26]. A title/abstract search, on the other hand, will provide numerous false positives in which the main focus is not on insulin resistance per se.

Bibliometric analysis

As described in previous studies, the bibliometric technique was applied[27-30]. The following bibliometric indicators were generated when the refined findings were exported to Microsoft Excel: (1) Growth pattern; (2) Type of publications; (3) Core countries; (4) Core institutions; (5) Core funding agencies; (6) Prolific authors; (7) Core journals with their impact factors (IF); and (8) Top 10 cited articles. The Impact Index per article for the top 10 highly-cited papers collected from *Reference Citation Analysis*, https://www.referencecitationanalysis.com, was presented. *Reference Citation Analysis* is an open, multidisciplinary citation analysis database owned by Baishideng Publishing Group Inc. (Pleasanton, CA 94566, United States)[31].

Visualized analysis

VOSviewer 1.6.18 was used to perform a co-occurrence analysis and visualize the collaborative networks of the countries to determine a worldwide scientific cooperation network across countries/regions and keywords in the titles and/or abstracts to determine hotspots and research trends. VOSviewer maps have nodes or frames that are colored and scaled differently. The node or the frame size is proportional to the number of times it appears. The node's or the frame's color indicates its link to other nodes with similar colors[32].

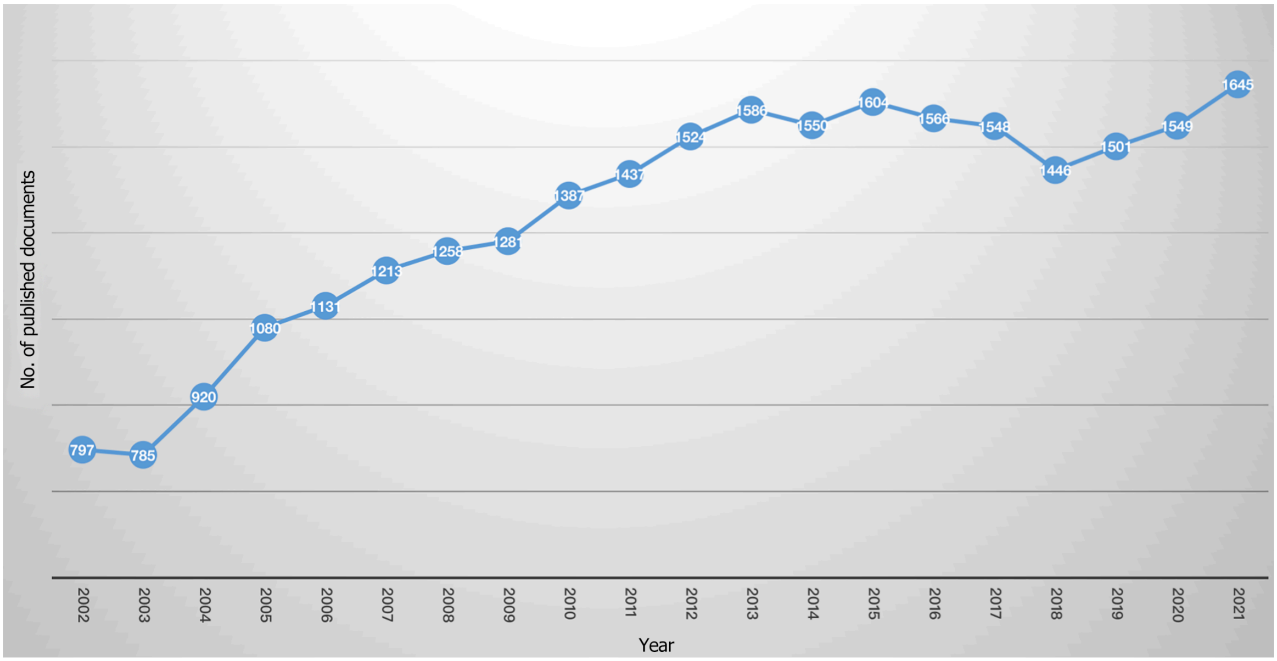

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Figure 1 Annual growth of publications on insulin resistance research the last two decades (2002-2021). Source: Own elaboration, based on Scopus; this figure created using EXCEL version 2013.

RESULTS

Current status and annual trend

A total of 26808 articles on insulin resistance were included in the Scopus database. The articles included research articles (n = 21918; 81.76%), review articles (n = 2641; 9.85%), and letters (n = 653; 2.44%). After 2003, as shown in Figure 1, the number of publications on insulin resistance studies increased rapidly. In 2021, 1645 papers were published, the highest amount in two decades.

Analysis of countries

During the study period, 136 countries contributed to research on insulin resistance. The highest number of articles was from the United States (n = 7360; 27.45%), followed by China (n = 3713; 13.85%), Japan (n = 1730, 6.45%), Italy (n = 1545; 5.54%), and the United Kingdom (n = 1484; 5.54%) (Table 1). The country network map included 42 frames (Figure 2). The top three countries in terms of centrality were the United States, China, and the United Kingdom. The centrality proved that they had close relationships and substantial intellectual effects on other countries.

Analysis of institutions

The top 10 active institutions are listed in Table 2. Harvard Medical School was first with 515 (1.92%) articles, followed by INSERM with 451 (1.68%) articles and the National Institutes of Health with 298 (1.11%). The top 10 active institutions were mainly based in the United States.

Analysis of funding agencies

Table 3 lists the top 10 funding agencies with the highest output. Seven funding agencies are from the United States, and one each is from Japan, China, and Canada. These countries contributed 10459 (39.01%) documents. The three most productive funding agencies were the National Institute of Diabetes and Digestive and Kidney Diseases (n = 2548; 9.50%), the National Institutes of Health (n = 2094, 7.81%), and National Heart, Lung, and Blood Institute (n = 1140, 4.25%).

Analysis of journals

Table 4 shows the top 10 most active journals. Diabetes Journal was first (n = 830; 3.10%), followed by Clinical Endocrinology and Metabolism (n = 692, 2.58%) and Diabetes Care (n = 623; 2.32%). Four of the journals on the active list were on the subject of diabetes. All the journals on the active list have a relatively high impact factor.

Analysis of citations

Table 5 lists the top 10 articles that were the most cited in research related to insulin resistance from 2002

Table 1 Top 10 most productive countries on insulin resistance research, ranked by the total number of publications in the last two decades (2002-2021)

Ranking	Country	Number of documents	%
1st	United States	7360	27.45
2nd	China	3713	13.85
3rd	Japan	1730	6.45
4th	Italy	1545	5.76
5th	United Kingdom	1484	5.54
6th	Canada	1186	4.42
7th	Germany	1070	3.99
8th	Spain	1061	3.96
9th	South Korea	1056	3.94
10th	France	858	3.20

Table 2 Top 10 most productive institutions in insulin resistance research, ranked by the total number of publications in the last two decades (2002-2021)

Ranking	Institute	Country	*n*	%
1st	Harvard Medical School	United States	515	1.92
2nd	INSERM	France	451	1.68
3rd	National Institutes of Health	United States	298	1.11
4th	University of Toronto	Canada	286	1.07
5th	Københavns Universitet	Denmark	280	1.04
6th	Karolinska Institutet	Sweden	268	1.00
7th	Consiglio Nazionale delle Ricerche	Italy	263	0.98
8th	VA Medical Center	United States	253	0.94
9th	Universidade de São Paulo	Brazil	247	0.92
10th	Yale School of Medicine	United States	234	0.87

to 2021. The 10 highest citations ranged from 4911 to 1827[33-42]. Furthermore, the 10 most cited articles have an impact index per article of 101.5 to 241.2 (Table 5).

Term co-occurrence cluster analysis of research hotspots

The term co-occurrence analysis provided a complete summary of hot topics discussed in insulin resistance research. VOSviewer detected 456 keywords that appeared a minimum of 300 times in the titles and abstracts of the included articles by analyzing the contents of the titles and abstracts. All terms were sorted into clusters on the VOSviewer keyword co-occurrence visualization map, and various clusters were colored differently (Figure 3). There are two clusters: (1) Cluster #1, shown by green dots, contained phrases typically found in publications relating to "inflammatory mechanisms in the regulation of insulin resistance"; and (2) Cluster #2, shown by red dots, contained phrases typically found in publications relating to "mechanisms linking obesity to insulin resistance". Hotspots in the field of insulin resistance were revealed *via* an overlay visualization map scaled by occurrence. The colored terms differ depending on when they appeared in the literature. The blue keywords were first shown, followed by the yellow keywords. After 2013, the most popular terms were related to inflammatory mechanisms in the regulation of insulin resistance (Figure 4).

Analysis of authorship

The total number of authors who participated in the publication of the retrieved documents was 80932, a mean of 3.1 authors per document. The list of the top 10 active authors in insulin resistance research, ranked by the total number of publications in the last two decades (2002-2021), is shown in Table 6. The top 10 list included four from the United States, three from Germany, two from Spain, and one from Italy.

Table 3 The top 10 funding agencies having the most publications on insulin resistance, ranked by the total number of publications in the last two decades (2002-2021)

Ranking	Institute	Country	*n*	%
1st	National Institute of Diabetes and Digestive and Kidney Diseases	United States	2548	9.50
2nd	National Institutes of Health	United States	2094	7.81
3rd	National Heart, Lung, and Blood Institute	United States	1140	4.25
4th	National Natural Science Foundation of China	China	1137	4.24
5th	National Center for Research Resources	United States	1051	3.92
6th	United States Department of Health and Human Services	United States	629	2.35
7th	National Institute on Aging	Canada	521	1.94
8th	Japan Society for the Promotion of Science	Japan	466	1.74
9th	National Center for Advancing Translational Sciences	United States	450	1.68
10th	Eunice Kennedy Shriver National Institute of Child Health and Human Development	United States	423	1.58

Table 4 Top 10 most productive journals on insulin resistance research, ranked by the total number of publications in the last two decades (2002-2021)

Ranking	Journal	*n*	%	IF[1]
1st	Diabetes	830	3.10	9.461
2nd	Journal of Clinical Endocrinology and Metabolism	692	2.58	5.958
3rd	Diabetes Care	623	2.32	19.112
4th	Plos One	517	1.93	3.2400
5th	Diabetologia	499	1.86	10.122
6th	Clinical and Experimental	425	1.59	8.694
7th	American Journal of Physiology Endocrinology and Metabolism	377	1.41	4.310
8th	Diabetes Research and Clinical Practice	227	0.85	5.602
9th	Obesity	219	0.82	5.002
10th	Scientific Reports	218	0.81	4.379

[1]2020 Journal Citation Reports® Science Edition (Clarivate Analytics, 2021). IF: Impact factor

DISCUSSION

Bibliometric analysis of insulin resistance publications in the last 20 years revealed that the number of articles published has gradually increased in recent years, indicating that more and more researchers are becoming involved in insulin resistance research. To our knowledge, this is the first bibliometric study that comprehensively examined worldwide trends in insulin resistance research over the last 20 years. The current study showed that research activity on insulin resistance was worldwide and involved countries in different world regions. The United States and China had a noticeable edge on this topic, probably due to a greater economy and investment in the scientific field. The research output from these countries may be related to a diverse spectrum of researchers interested in this topic and strong financial support for researchers.

Another important reason for the contribution of different world regions is the high level of international collaboration, as evident from the thick lines coming out from most countries in the visualization map. This collaboration was initiated because different regions of the research groups in different regions of the world were involved in different aspects of insulin resistance research or different complications of insulin resistance. Another area of relevance for the current study with regard to scientific publications on insulin resistance is the quality of research papers. It is worth noting that nine of the top 10 cited articles were published in journals with an IF larger than 10, implying that they have a large impact in medicine: Journal of Clinical Investigation, Cell Metabolism, and Nature. As shown, articles related to insulin resistance have been published both in endocrinology and non-endocrinology

Table 5 Top 10 most cited papers on research related to insulin resistance, ranked by the total number of citations in the last two decades (2002-2021)

Ranking	Ref.	Journal name	Cited by	IF[1]	Impact index per article[2]	Type of paper
1st	Xu *et al*[36], 2003	Journal of Clinical Investigation	4911	14.808	241.2	Original article
2nd	Cani *et al*[40], 2007	Diabetes	3645	9.461	222.2	Original article
3rd	Kahn *et al*[39], 2006	Nature	3109	49.962	185.2	Review articles
4th	Shoelson *et al*[37], 2006	Journal of Clinical Investigation	2822	14.808	156.4	Review articles
5th	Shi *et al*[42], 2006	Journal of Clinical Investigation	2521	14.808	149.0	Original article
6th	Hirosumi *et al*[35], 2002	Nature	2503	49.962	112.6	Letter to the editor
7th	Kadowaki *et al*[33], 2006	Journal of Clinical Investigation	2140	14.808	112.9	Review articles
8th	Newgard *et al*[34], 2009	Cell Metabolism	1852	27.787	139.7	Original article
9th	Houstis *et al*[41], 2006	Nature	1838	49.962	101.5	Letter to the editor
10th	Kanda *et al*[38], 2006	Journal of Clinical Investigation	1827	14.808	105.4	Original article

[1]2020 Journal Citation Reports® Science Edition (Clarivate Analytics, 2021).
[2]The Impact Index Per Article is presented based on *Reference Citation Analysis*, https://www.referencecitationanalysis.com [Source: Baishideng Publishing Group Inc (Pleasanton, CA 94566, United States)]. IF: Impact factor.

Table 6 List of top 10 active authors in insulin resistance research, ranked by the total number of publications in the last two decades (2002-2021)

Ranking	Author	Country	*n*	%	*H* index
1st	Shulman GI	United States	150	0.56	154
2nd	Haffner SM	United States	86	0.32	144
3rd	Reaven GM	United States	76	0.28	120
3rd	Roden M	Germany	76	0.28	86
5th	Häring HU	Germany	75	0.28	104
6th	Fritsche A	Germany	70	0.26	80
7th	Fernández-Real JM	Spain	68	0.25	75
7th	Izaola O	Spain	68	0.25	32
7th	Wagenknecht LE	United States	68	0.25	87
10th	Pacini G	Italy	65	0.24	65

subject areas, such as medicine, biochemistry, genetics, and molecular biology, nursing, pharmacology, toxicology, and pharmaceutics, agricultural and biological sciences, neuroscience, and immunology and microbiology journals, revealing the contribution and collaboration of many researchers from different subject areas. Previous research has confirmed that[43-45]. The findings of this study confirm the close association between IF and citations and the fact that the most cited articles are frequently published in journals at the top of the IF list, which helps these journals maintain their high IF.

Furthermore, the increase in insulin resistance publications can be attributed to the fact that numerous hot topics were published during this period[33-37], exposing novel hypotheses and establishing new research fields such as "inflammatory mechanisms in the regulation of insulin resistance" and "mechanisms linking obesity and insulin resistance". Several studies have shown that inflammation is a critical mediator in obesity-induced insulin resistance. Most of these investigations examined the links between adipose tissue in obesity and the regulation of inflammation and insulin resistance[46-49] and the mechanisms by which dietary anti-inflammatory components/functional nutrients may be helpful[50-52].

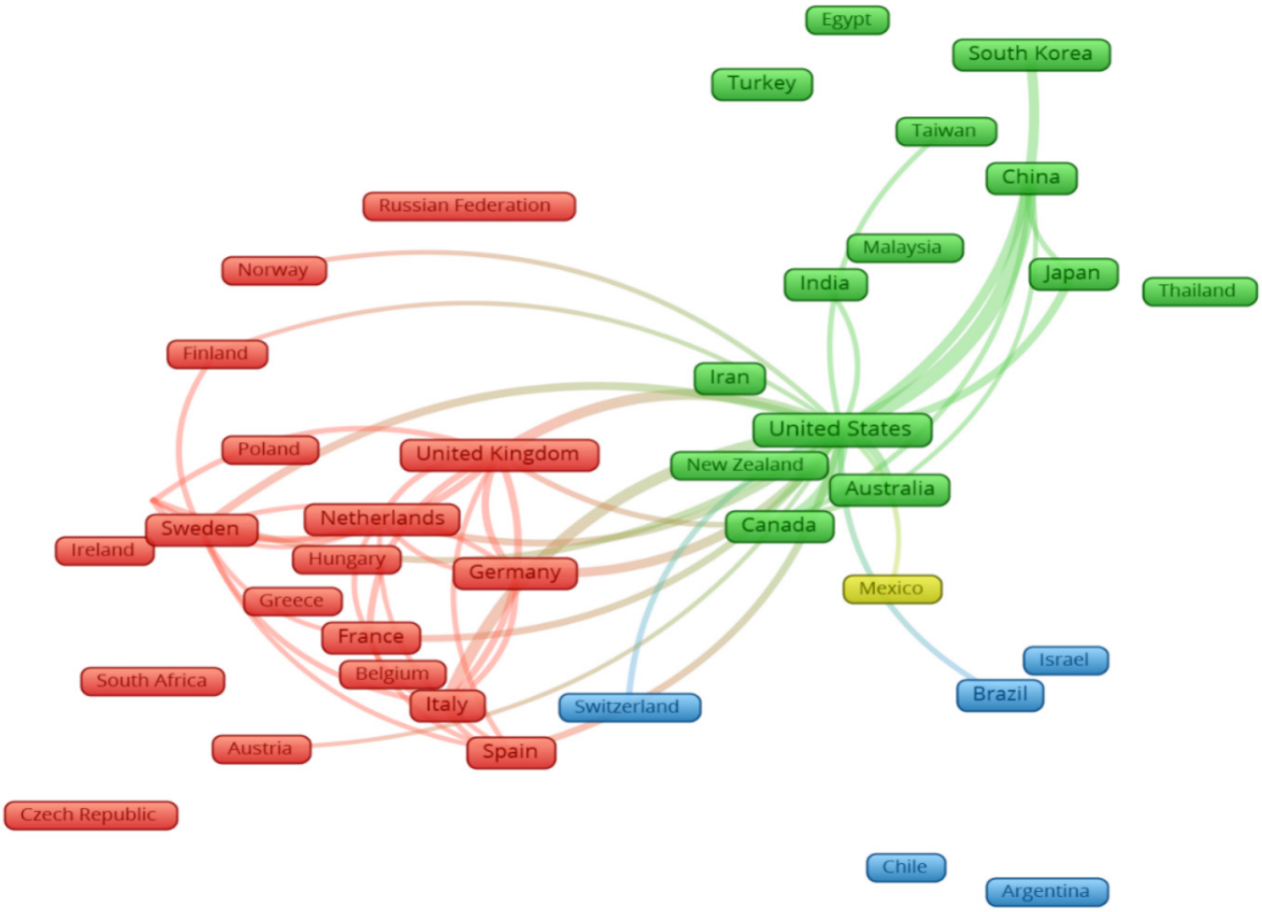

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Figure 2 Map of visualization of worldwide research collaboration network. Countries with short distances and extensive connecting lines had a significant research collaboration. This collaborative map was built when each country had at least 100 articles. Source: Own elaboration, based on Scopus database; figure created using VOSviewer Software.

Publications with the highest citation frequencies have the greatest academic effect[53,54]. For example, the study published in the Journal of Clinical Investigation in 2003 by Xu *et al*[36] was ranked first. It was revealed that macrophages in white adipose tissue are involved in morbid obesity and that macrophage-associated inflammatory activities may contribute to the pathophysiology of obesity-induced insulin resistance[36]. The article ranked second was published in Diabetes by Cani *et al*[40]. Metabolic endotoxemia was found to alter the inflammatory tone of the body, causing weight gain and diabetes[40].

Strengths and limitations

This is the first bibliometric and visual analysis study to investigate research trends and hotspots in insulin resistance from 2002 to 2021. The current study reviewed linked papers on this issue from numerous perspectives, demonstrated a comprehensive view of understanding in this field during the last few years, and gave direction for future investigations. New researchers in this discipline may simply access meaningful and relevant material with the aid of this bibliometric study. However, certain limitations apply to the generalizability of these findings. First, bibliometric analyses solely used published material from the Scopus database. This may underestimate the amount of research done in South America, China, the Middle East, and other regions of the globe with non-English and unindexed publications. Second, because bibliometric data changes over time, indexing delays may have caused a slight (but not significant) in the number of documents or other metrics. Third, to avoid selection bias, the current study only searched the title for terms such as "insulin resistance" or "insulin sensitivity". As a result, the possibility of false positive or false negative results should always be considered. Fourth, Scopus's results reflect the type and content of Scopus's database. As a result, if prolific authors have two or more Scopus profiles, their research output is likely to be dispersed, and their names may not appear in the active list. The same is true when alternative spellings of an institution's name are used in published documents. As a result, interpreting data about the most active authors, institutions, and nations should be limited to the Scopus findings produced using the described technique.

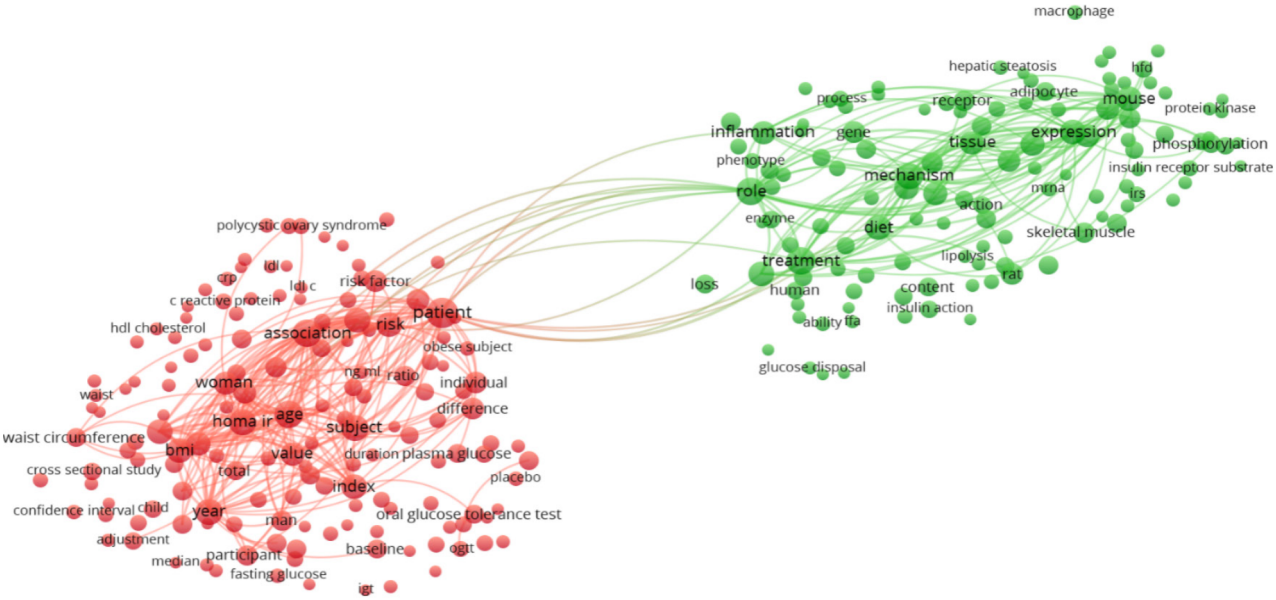

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Figure 3 Network visualization map of terms in the titles/abstracts with a minimum occurrence of 300 or more. Of the 250809 terms in this field, 456 achieved this threshold, were grouped into two clusters, and colored differently. Each cluster represents a general research theme present in the retrieved documents. Source: Own elaboration, based on Scopus database; figure created using VOSviewer Software. LDL-C: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein; IGT: Impaired glucose tolerance; OGTT: Oral glucose tolerance test; BMI: Body mass index; HFD: High fat diet; IRS: Insulin receptor substrate; CRP: C-reactive protein; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.

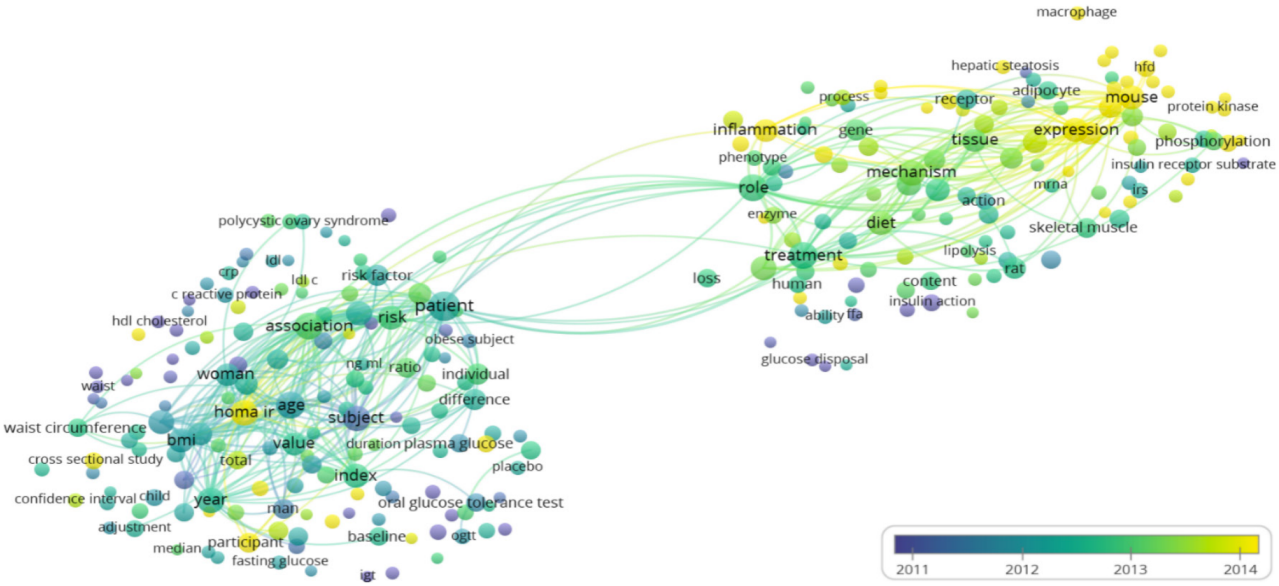

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Figure 4 Network visualization map of terms in the title/abstract according to the average timing of their appearance. Blue represents early appearance, and yellow represents late appearance. Source: Own elaboration, based on Scopus database; figure created using VOSviewer Software. LDL-C: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein; IGT: Impaired glucose tolerance; OGTT: Oral glucose tolerance test; BMI: Body mass index; HFD: High fat diet; IRS: Insulin receptor substrate; CRP: C-reactive protein; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.

CONCLUSION

To our knowledge, this was the first study to conduct a comprehensive bibliometric analysis of insulin resistance publications from 2002 to 2021, covering the publication year, the number of citations, and current hot topics and trends projected from them. Our data showed that insulin resistance has steadily gained interest from researchers, as evidenced by the number of citations and yearly publications. So far, the United States has been the undisputed leader in this topic, which cannot be divorced from adequate funding sources. Publications have grown significantly in the last decade, while low-income

countries with greater burdens continue to produce fewer publications in this field. "Inflammatory mechanisms in the regulation of insulin resistance" and "mechanisms linking obesity to insulin resistance" were hotspots for insulin resistance research in the past 20 years. This approach might assist researchers in choosing new research areas and recognizing research hotspots and frontiers. In the future, perhaps high-quality clinical evidence will be acquired.

ARTICLE HIGHLIGHTS

Research background

Insulin resistance is a condition in which muscle cells take up and store glucose and triglycerides, resulting in elevated amounts of glucose and triglycerides circulating in the bloodstream.

Research motivation

Several bibliometric studies have been carried out on the subject of diabetic investigation. However, no bibliometric study has been done on research into insulin resistance.

Research objectives

This bibliometric study aimed to identify and assess the current state and trends in insulin resistance research production worldwide and visually analyze research hotspots on this subject.

Research methods

The Scopus database and *Reference Citation Analysis* were used to compile the literature on insulin resistance. In addition, VOSviewer software was used to visually assess data collected from relevant publications.

Research results

This is the first bibliometric analysis of trends in insulin resistance. The number of publications on insulin resistance has increased in the last decade. Our results indicated that the "inflammatory mechanisms in the regulation of insulin resistance" and "mechanisms linking obesity to insulin resistance" will remain research hotspots in the future.

Research conclusions

Our findings indicate that interest in insulin resistance has gradually increased among researchers, as shown by the increasing number of citations and annual publications. Moreover, publications in this field have increased significantly in the last decade, while low-income countries with higher burdens continue to produce fewer publications.

Research perspectives

This paper contributes essential information by providing references and suggestions for future research on pathophysiology and clinical uses of insulin resistance. This approach may aid researchers in identifying new topics of inquiry and identifying research hotspots and frontiers. Perhaps in the future, high-quality clinical evidence will be collected.

FOOTNOTES

Author contributions: Zyoud SH developed the concept for the manuscript, reviewed the literature, designed the study, collected the data, analyzed the data, made significant contributions to the existing literature search and interpretation of the manuscript, and wrote the manuscript; Shakhshir M, Koni A, Abushanab AS, Jairoun AA, Shahwan WM, Al Subu R, Abu Taha A, and Al-Jabi SW participated in interpretation of the data and made revisions to the initial draft; and all authors provided critical review and approved the final manuscript before submission.

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REFERENCES

1 **World Health Organization**. Diabetes. [cited 5 February 2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes
2 **Taylor R**. Insulin resistance and type 2 diabetes. *Diabetes* 2012; **61**: 778-779 [PMID: 22442298 DOI: 10.2337/db12-0073]
3 **Freeman AM**, Pennings N. Insulin Resistance. 2022 Jul 4. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan- [PMID: 29939616]
4 **da Silva AA**, do Carmo JM, Li X, Wang Z, Mouton AJ, Hall JE. Role of Hyperinsulinemia and Insulin Resistance in Hypertension: Metabolic Syndrome Revisited. *Can J Cardiol* 2020; **36**: 671-682 [PMID: 32389340 DOI: 10.1016/j.cjca.2020.02.066]
5 **Song Y**, Zhao F. Bibliometric analysis of metabolic surgery for type 2 diabetes: current status and future prospects. *Updates Surg* 2022; **74**: 697-707 [PMID: 35094308 DOI: 10.1007/s13304-021-01201-5]
6 **Chen L**, Ma S, Hu D, Lin H, Zhu Y, Chen K, Chen L, Zheng C, Liu J, Liao Y. Bibliometric Study of Sodium Glucose Cotransporter 2 Inhibitors in Cardiovascular Research. *Front Pharmacol* 2020; **11**: 561494 [PMID: 33041801 DOI: 10.3389/fphar.2020.561494]
7 **Lin X**, Chen P, Lin F. Mapping global research trends in diabetes and COVID-19 outbreak in the past year: a bibliometric analysis. *Ann Palliat Med* 2022; **11**: 1241-1252 [PMID: 34806394 DOI: 10.21037/apm-21-2636]
8 **Dong Y**, Liu Y, Yu J, Qi S, Liu H. Mapping research trends in diabetic retinopathy from 2010 to 2019: A bibliometric analysis. *Medicine (Baltimore)* 2021; **100**: e23981 [PMID: 33545985 DOI: 10.1097/MD.0000000000023981]
9 **Hosseinkhani S**, Aazami H, Hashemi E, Dehghanbanadaki H, Adibi-Motlagh B, Razi F. The trend in application of omics in type 2 diabetes researches; A bibliometric study. *Diabetes Metab Syndr* 2021; **15**: 102250 [PMID: 34419857 DOI: 10.1016/j.dsx.2021.102250]
10 **Zou X**, Sun Y. Bibliometrics Analysis of the Research Status and Trends of the Association Between Depression and Insulin From 2010 to 2020. *Front Psychiatry* 2021; **12**: 683474 [PMID: 34366917 DOI: 10.3389/fpsyt.2021.683474]
11 **Bornmann L**. Measuring impact in research evaluations: a thorough discussion of methods for, effects of and problems with impact measurements. *High Educ* 2017; 73: 775-787 [DOI: 10.1007/s10734-016-9995-x]
12 **Gu D**, Li J, Li X, Liang C. Visualizing the knowledge structure and evolution of big data research in healthcare informatics. *Int J Med Inform* 2017; **98**: 22-32 [PMID: 28034409 DOI: 10.1016/j.ijmedinf.2016.11.006]
13 **Wang X**, Guo J, Gu D, Yang Y, Yang X, Zhu K. Tracking knowledge evolution, hotspots and future directions of emerging technologies in cancers research: a bibliometrics review. *J Cancer* 2019; **10**: 2643-2653 [PMID: 31258772 DOI: 10.7150/jca.32739]
14 **Sweileh WM**. Substandard and falsified medical products: bibliometric analysis and mapping of scientific research. *Global Health* 2021; **17**: 114 [PMID: 34556126 DOI: 10.1186/s12992-021-00766-5]
15 **Sweileh WM**. Global research activity on mathematical modeling of transmission and control of 23 selected infectious disease outbreak. *Global Health* 2022; **18**: 4 [PMID: 35062966 DOI: 10.1186/s12992-022-00803-x]
16 **Sweileh WM**. A bibliometric analysis of health-related literature on natural disasters from 1900 to 2017. *Health Res Policy Syst* 2019; **17**: 18 [PMID: 30744641 DOI: 10.1186/s12961-019-0418-1]
17 **Cebrino J**, Portero de la Cruz S. A worldwide bibliometric analysis of published literature on workplace violence in healthcare personnel. *PLoS One* 2020; **15**: e0242781 [PMID: 33227018 DOI: 10.1371/journal.pone.0242781]
18 **Sweileh WM**. Bibliometric analysis of global scientific literature on vaccine hesitancy in peer-reviewed journals (1990-2019). *BMC Public Health* 2020; **20**: 1252 [PMID: 32807154 DOI: 10.1186/s12889-020-09368-z]
19 **Su KZ**, Li YR, Zhang D, Yuan JH, Zhang CS, Liu Y, Song LM, Lin Q, Li MW, Dong J. Relation of Circulating Resistin to Insulin Resistance in Type 2 Diabetes and Obesity: A Systematic Review and Meta-Analysis. *Front Physiol* 2019; **10**: 1399 [PMID: 31803062 DOI: 10.3389/fphys.2019.01399]
20 **Sampath Kumar A**, Maiya AG, Shastry BA, Vaishali K, Ravishankar N, Hazari A, Gundmi S, Jadhav R. Exercise and insulin resistance in type 2 diabetes mellitus: A systematic review and meta-analysis. *Ann Phys Rehabil Med* 2019; **62**: 98-103 [PMID: 30553010 DOI: 10.1016/j.rehab.2018.11.001]
21 **Shoshtari-Yeganeh B**, Zarean M, Mansourian M, Riahi R, Poursafa P, Teiri H, Rafiei N, Dehdashti B, Kelishadi R. Systematic review and meta-analysis on the association between phthalates exposure and insulin resistance. *Environ Sci Pollut Res Int* 2019; **26**: 9435-9442 [PMID: 30734259 DOI: 10.1007/s11356-019-04373-1]
22 **Sweileh WM**. Global research activity on antimicrobial resistance in food-producing animals. *Arch Public Health* 2021; **79**: 49 [PMID: 33849636 DOI: 10.1186/s13690-021-00572-w]
23 **Sweileh WM**. Bibliometric analysis of peer-reviewed literature on antimicrobial stewardship from 1990 to 2019. *Global Health* 2021; **17**: 1 [PMID: 33397377 DOI: 10.1186/s12992-020-00651-7]
24 **Sweileh WM**. Health-related publications on people living in fragile states in the alert zone: a bibliometric analysis. *Int J Ment Health Syst* 2020; **14**: 70 [PMID: 32868982 DOI: 10.1186/s13033-020-00402-6]

25 **Sweileh WM**. Global research publications on systemic use of off-label and unlicensed drugs: A bibliometric analysis (1990-2020). *Int J Risk Saf Med* 2022; **33**: 77-89 [PMID: 34275912 DOI: 10.3233/JRS-210012]

26 **Sweileh WM**. Global Research Activity on Elder Abuse: A Bibliometric Analysis (1950-2017). *J Immigr Minor Health* 2021; **23**: 79-87 [PMID: 32488667 DOI: 10.1007/s10903-020-01034-1]

27 **Abushamma F**, Barqawi A, Al-Jabi SW, Akkawi M, Maree M, Zyoud SH. Global Analysis of Research Trends on Kidney Function After Nephron-Sparing Surgery: A Bibliometric and Visualised Study. *Cancer Manag Res* 2021; **13**: 7479-7487 [PMID: 34611441 DOI: 10.2147/CMAR.S324284]

28 **Zyoud SH**, Smale S, Waring WS, Sweileh W, Al-Jabi SW. Global research trends in the microbiome related to irritable bowel syndrome: A bibliometric and visualized study. *World J Gastroenterol* 2021; **27**: 1341-1353 [PMID: 33833487 DOI: 10.3748/wjg.v27.i13.1341]

29 **Barqawi A**, Abushamma FA, Akkawi M, Al-Jabi SW, Shahwan MJ, Jairoun AA, Zyoud SH. Global trends in research related to sleeve gastrectomy: A bibliometric and visualized study. *World J Gastrointest Surg* 2021; **13**: 1509-1522 [PMID: 34950437 DOI: 10.4240/wjgs.v13.i11.1509]

30 **Zyoud SH**, Al-Jabi SW. Mapping the situation of research on coronavirus disease-19 (COVID-19): a preliminary bibliometric analysis during the early stage of the outbreak. *BMC Infect Dis* 2020; **20**: 561 [PMID: 32738881 DOI: 10.1186/s12879-020-05293-z]

31 **Baishideng Publishing Group Inc**. Reference Citation Analysis. [cited 2 February 2022]. Available from: https://www.referencecitationanalysis.com

32 **van Eck NJ**, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 2010; **84**: 523-538 [PMID: 20585380 DOI: 10.1007/s11192-009-0146-3]

33 **Kadowaki T**, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006; **116**: 1784-1792 [PMID: 16823476 DOI: 10.1172/JCI29126]

34 **Newgard CB**, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS Jr, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 2009; **9**: 311-326 [PMID: 19356713 DOI: 10.1016/j.cmet.2009.02.002]

35 **Hirosumi J**, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333-336 [PMID: 12447443 DOI: 10.1038/nature01137]

36 **Xu H**, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003; **112**: 1821-1830 [PMID: 14679177 DOI: 10.1172/JCI19451]

37 **Shoelson SE**, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; **116**: 1793-1801 [PMID: 16823477 DOI: 10.1172/JCI29069]

38 **Kanda H**, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 2006; **116**: 1494-1505 [PMID: 16691291 DOI: 10.1172/JCI26498]

39 **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]

40 **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850 DOI: 10.2337/db06-1491]

41 **Houstis N**, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006; **440**: 944-948 [PMID: 16612386 DOI: 10.1038/nature04634]

42 **Shi H**, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 2006; **116**: 3015-3025 [PMID: 17053832 DOI: 10.1172/JCI28898]

43 **Tas F**. An analysis of the most-cited research papers on oncology: which journals have they been published in? *Tumour Biol* 2014; **35**: 4645-4649 [PMID: 24414487 DOI: 10.1007/s13277-014-1608-7]

44 **Sharma M**, Sarin A, Gupta P, Sachdeva S, Desai AV. Journal impact factor: its use, significance and limitations. *World J Nucl Med* 2014; **13**: 146 [PMID: 25191134 DOI: 10.4103/1450-1147.139151]

45 **Saha S**, Saint S, Christakis DA. Impact factor: a valid measure of journal quality? *J Med Libr Assoc* 2003; **91**: 42-46 [PMID: 12572533]

46 **Olefsky JM**, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* 2010; **72**: 219-246 [PMID: 20148674 DOI: 10.1146/annurev-physiol-021909-135846]

47 **Vandanmagsar B**, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med* 2011; **17**: 179-188 [PMID: 21217695 DOI: 10.1038/nm.2279]

48 **Dandona P**, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004; **25**: 4-7 [PMID: 14698276 DOI: 10.1016/j.it.2003.10.013]

49 **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]

50 **McArdle MA**, Finucane OM, Connaughton RM, McMorrow AM, Roche HM. Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. *Front Endocrinol (Lausanne)* 2013; **4**: 52 [PMID: 23675368 DOI: 10.3389/fendo.2013.00052]

51 **Maeda N**, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002; **8**: 731-737 [PMID: 12068289 DOI: 10.1038/nm724]

52 **Vrieze A**, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; **143**: 913-6.e7 [PMID: 22728514 DOI: 10.1053/j.gastro.2012.06.031]

53 **Filion KB**, Pless IB. Factors related to the frequency of citation of epidemiologic publications. *Epidemiol Perspect Innov* 2008; **5**: 3 [PMID: 18302781 DOI: 10.1186/1742-5573-5-3]

54 **Opthof T**. Differences in citation frequency of clinical and basic science papers in cardiovascular research. *Med Biol Eng Comput* 2011; **49**: 613-621 [PMID: 21567267 DOI: 10.1007/s11517-011-0783-6]

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LETTER TO THE EDITOR

Different nutrient compositions in diet and taking hypoglycemic drugs can modulate gut microbial flora

Zi-Jun Lin, Qiong-Wen Zhang, Xiao-Lan Yu, Bo Zhou, Chang-Wei Liu, Lian-Ping He

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Abstract

The diet structure of diabetic patients is different from that of normal people. Diabetic patients also need to take hypoglycemic drugs to regulate blood sugar. Both dieting and drugs affect the gut microbiota of diabetic patients. In this letter, we discuss that different dietary patterns and the use of hypoglycemic agents may have an impact on changes in gut microbiota in diabetic patients.

Key Words: Diabetic patients; Gut microbiota; Hypoglycemic drugs

Core Tip: Changes in diet can lead to changes in the composition of gut microbiota in diabetic patients. On the other hand, taking hypoglycemic drugs can also change the gut microbiota. Therefore, it is necessary to consider the dietary structure and the use of hypoglycemic drugs in the study of changes in the intestinal flora of patients with diabetes.

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TO THE EDITOR

Diabetes mellitus (DM) is one of important risk factor for population health in the twenty-first century worldwide. It is of great significance to explore the lifestyle intervention mode for the prevention and treatment of type 2 diabetes mellitus (T2DM). One study found intermittent hypoxia (IH) was associated with metabolic diseases including as obesity and obstructive sleep apnea-hypopnea syndrome (OSAHS)[1]. IH may be involved in selective alterations of the gut microbiota of T2DM patients with OSAHS. Similarly, changes in gut microbiota can affect the development of T2DM.

Gut microbiota is known to change with diet. Especially when someone suffering from T2DM, doctors often recommend dietary changes to curb the progression of the disease. Changes in eating habits can disrupt the balance of gut microbiota when the body's resistance is low[2]. The study by Liu *et al*[3] showed that blood sugar levels in mouse model of T2DM induced by streptozotocin-high-fat diet were changes with gut microbiota. A large number of studies have shown that diet affects the development of diabetes. For example, blackcurrant extract enhanced insulin sensitivity and glucose-stimulated insulin secretion in non-obese type 2 diabetic rats[4]. Therefore, the diet structure will affect the gut microbiota. However, the following questions need to be further clarified in future studies. What nutrition considerations for persons with diabetes that will impact persons? What are the recommendations for persons with diabetes concerning nutrition to be considered? The link between gut microbial, diabetes, nutrition and autoimmunity are important, but still need to be addressed.

In addition, hypoglycemic drugs also affect gut microbiota. In this study, drugs that regulate IH have an effect on the balance of gut microbiota. We strongly agree with this view. However, when taking drugs to regulate IH, hypoglycemic drugs are also used. Therefore, hypoglycemic drugs also have an impact on the balance of gut microbiota. For some patients who require combination therapy to treat diabetes and complications of diabetes, especially after combination therapy with antibiotics and hypoglycemic drugs, the impact on the intestinal flora is significant[5]. Clinically, metformin is widely used in the treatment of T2DM. Studies have shown that gut microbiota is an active site of metformin. The gut is a potential target of metformin. Metformin induce butyrate and propionate involving glucose homeostasis[6]. When diabetics take metformin, the gut microbiota will definitely change. Studies have also shown that treating diabetic mice with oleuropein (OP) is also treated by modulating the gut microbiota. The OP could decrease fasting blood glucose levels and improve glucose tolerance[7]. Hypoglycemic drugs are also taken when taking drugs that regulate IH, so hypoglycemic drugs will also affect the intestinal flora of patients with IH. It is common for IH patients to take hypoglycemic drugs, so the regulation of hypoglycemic drugs on gut microbiota also needs to be discussed.

On the other hand, IH may be associated with changes in gut microbiota, and it is possible that changes in gut microbiota led to IH. In the clinical setting, the effective treatment for IH is usually oxygen therapy. Thus, we can use oxygen inhalation to intervene in changes in gut microbiota. By conducting oxygen supply experiments, the relationship between IH and gut microbiota can be further reflected, which makes the research results more convincing.

The diet structure of diabetic patients is different from that of normal people. Diabetic patients also need to take hypoglycemic drugs to regulate blood sugar. Both dietary structure of patients with diabetes and hypoglycemic drugs taken by the patients with diabetes can alter the gut microbiota of the patients. Therefore, the influence of diet and drugs on the gut microbiota cannot be ignored.

FOOTNOTES

Author contributions: He LP and Liu CW contributed to the conception of research; Zhang QW, Lin ZJ and Zhou B wrote the manuscript; Zhang QW, Lin ZJ and Yu XL contributed to the revision of the manuscript.

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REFERENCES

1 **Tang SS**, Liang CH, Liu YL, Wei W, Deng XR, Shi XY, Wang LM, Zhang LJ, Yuan HJ. Intermittent hypoxia is involved in gut microbial dysbiosis in type 2 diabetes mellitus and obstructive sleep apnea-hypopnea syndrome. *World J Gastroenterol* 2022; **28**: 2320-2333 [PMID: 35800187 DOI: 10.3748/wjg.v28.i21.2320]

2 **Song M**, Chan AT, Sun J. Influence of the Gut Microbiome, Diet, and Environment on Risk of Colorectal Cancer. *Gastroenterology* 2020; **158**: 322-340 [PMID: 31586566 DOI: 10.1053/j.gastro.2019.06.048]

3 **Liu S**, Qin P, Wang J. High-Fat Diet Alters the Intestinal Microbiota in Streptozotocin-Induced Type 2 Diabetic Mice. *Microorganisms* 2019; **7** [PMID: 31208113 DOI: 10.3390/microorganisms7060176]

4 **Yang HJ**, Zhang T, Wu XG, Kim MJ, Kim YH, Yang ES, Yoon YS, Park S. Aqueous Blackcurrant Extract Improves Insulin Sensitivity and Secretion and Modulates the Gut Microbiome in Non-Obese Type 2 Diabetic Rats. *Antioxidants (Basel)* 2021; **10** [PMID: 34068659 DOI: 10.3390/antiox10050756]

5 **Zarrinpar A**, Chaix A, Xu ZZ, Chang MW, Marotz CA, Saghatelian A, Knight R, Panda S. Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nat Commun* 2018; **9**: 2872 [PMID: 30030441 DOI: 10.1038/s41467-018-05336-9]

6 **Wu H**, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, Ståhlman M, Olsson LM, Serino M, Planas-Fèlix M, Xifra G, Mercader JM, Torrents D, Burcelin R, Ricart W, Perkins R, Fernàndez-Real JM, Bäckhed F. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med* 2017; **23**: 850-858 [PMID: 28530702 DOI: 10.1038/nm.4345]

7 **Zheng S**, Wang Y, Fang J, Geng R, Li M, Zhao Y, Kang SG, Huang K, Tong T. Oleuropein Ameliorates Advanced Stage of Type 2 Diabetes in *db*/*db* Mice by Regulating Gut Microbiota. *Nutrients* 2021; **13** [PMID: 34206641 DOI: 10.3390/nu13072131]

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