ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2021 June 15; 12(6): 685-915

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 12 Number 6 June 15, 2021

REVIEW

MINIREVIEWS

World Journal of Diabetes

Monthly Volume 12 Number 6 June 15, 2021

Contents

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Jue Liu, MD, PhD, Associated Professor, Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing 100191, China. jueliu@bjmu.edu.cn

AIMS AND SCOPE

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

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

INDEXING/ABSTRACTING

The *WJD* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Current Contents/Clinical Medicine, Journal Citation Reports/Science Edition, PubMed, and PubMed Central. The 2020 Edition of Journal Citation Reports® cites the 2019 impact factor (IF) for *WJD* as 3.247; IF without journal self cites: 3.222; Ranking: 70 among 143 journals in endocrinology and metabolism; and Quartile category: Q2.

RESPONSIBLE EDITORS FOR THIS ISSUE

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

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Timothy Koch

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

PUBLICATION DATE
June 15, 2021

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 685-705

DOI: 10.4239/wjd.v12.i6.685

ISSN 1948-9358 (online)

REVIEW

Role of novel biomarkers in diabetic cardiomyopathy

Marko Kumric, Tina Ticinovic Kurir, Josip A Borovac, Josko Bozic

ORCID number: Marko Kumric 0000-0002-9696-3359; Tina Ticinovic Kurir 0000-0003-1695-9235; Josip A Borovac 0000-0002-4878-8146; Josko Bozic 0000-0003-1634-0635.

Author contributions: Kumric M, Ticinovic Kurir T and Bozic J for conceptualization, original draft preparation, and supervision; Kumric M and Borovac JA for review of literature and visualization; All authors contributed to the final draft of the manuscript; all authors have read and agreed to the published version of the manuscript.

Conflict-of-interest statement: We report no conflicts of interest.

Manuscript source: Invited manuscript

Marko Kumric, Tina Ticinovic Kurir, Josip A Borovac, Josko Bozic, Department of Pathophysiology, University of Split School of Medicine, Split 21000, Croatia

Tina Ticinovic Kurir, Department of Endocrinology, University Hospital of Split, Split 21000, Croatia

Josip A Borovac, Emergency Medicine, Institute of Emergency Medicine of Split-Dalmatia County, Split 21000, Croatia

Corresponding author: Josko Bozic, MD, PhD, Associate Professor, Department of Pathophysiology, University of Split School of Medicine, Soltanska 2, Split 21000, Croatia. josko.bozic@mefst.hr

Abstract

Diabetic cardiomyopathy (DCM) is commonly defined as cardiomyopathy in patients with diabetes mellitus in the absence of coronary artery disease and hypertension. As DCM is now recognized as a cause of substantial morbidity and mortality among patients with diabetes mellitus and clinical diagnosis is still inappropriate, various expert groups struggled to identify a suitable biomarker that will help in the recognition and management of DCM, with little success so far. Hence, we thought it important to address the role of biomarkers that have shown potential in either human or animal studies and which could eventually result in mitigating the poor outcomes of DCM. Among the array of biomarkers we thoroughly analyzed, long noncoding ribonucleic acids, soluble form of suppression of tumorigenicity 2 and galectin-3 seem to be most beneficial for DCM detection, as their plasma/serum levels accurately correlate with the early stages of DCM. The combination of relatively inexpensive and accurate speckle tracking echocardiography with some of the highlighted biomarkers may be a promising screening method for newly diagnosed diabetes mellitus type 2 patients. The purpose of the screening test would be to direct affected patients to more specific confirmation tests. This perspective is in concordance with current guidelines that accentuate the importance of an interdisciplinary team-based approach.

Key Words: Diabetic cardiomyopathy; Heart failure; Biomarkers; Diabetes mellitus; Cardiomyopathy

Specialty type: Cardiac and cardiovascular systems

Country/Territory of origin: Croatia

Peer-review report's scientific quality classification

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

Received: December 28, 2020
Peer-review started: December 28, 2020
First decision: January 11, 2021
Revised: January 22, 2021
Accepted: March 18, 2021
Article in press: March 18, 2021
Published online: June 15, 2021

P-Reviewer: Shen XC, Zhang DM
S-Editor: Zhang L
L-Editor: Webster JR
P-Editor: Ma YJ

Core Tip: Diabetic cardiomyopathy (DCM), which affects 12% of diabetics, is an under-recognized and lethal complication of diabetes. Thus, there is an urgent need for reliable and available biomarkers for DCM detection. To date, none of the conducted studies have been successful in identifying such biomarkers. Hence, in concordance with current guidelines that accentuate the importance of an interdisciplinary team-based approach, we propose the combination of speckle tracking echocardiography and a few novel biomarkers as a screening method for DCM in patients with new onset diabetes mellitus type 2.

Citation: Kumric M, Ticinovic Kurir T, Borovac JA, Bozic J. Role of novel biomarkers in diabetic cardiomyopathy. *World J Diabetes* 2021; 12(6): 685-705
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/685.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.685

INTRODUCTION

The first records of diabetic cardiomyopathy (DCM) date back to 1972[1], when it was first observed in the post-mortem analysis of diabetics who died of heart failure (HF), having no evidence of coronary artery pathology or any other pathology that could explain the observed structural changes. These findings were supported by the Framingham study in which HF was five times more common among patients with diabetes mellitus (DM)[2], even after the adjustment for hypertension and coronary heart disease. DCM is now commonly defined as cardiomyopathy in patients with DM in the absence of coronary artery disease, valvular disease, and hypertension, or any other conventional cardiovascular risk factor for that matter[3]. Diagnostic criteria include left ventricular diastolic dysfunction and/or reduced left ventricular ejection fraction, pathological left ventricle hypertrophy and interstitial fibrosis[4]. However, timely and appropriate diagnosis is still fairly challenging in everyday clinical practice [5]. The reason behind the exigent diagnosis of this clinical entity lies in the long asymptomatic phase of the disease. DCM initially presents with clinically covert myocardial fibrosis, dysfunctional cardiac remodeling and associated diastolic dysfunction, later progressing to systolic dysfunction, and eventually to overt HF. The changes that lead to DCM are triggered by hyperinsulinemia and increased insulin resistance, whereas the underlying molecular changes that are involved in the pathophysiologic development of DCM include: Abnormalities in the adenosine monophosphate-activated protein kinase, nuclear factor κ-light-chain-enhancer of activated B cells (NFκB), nuclear factor erythroid 2–related factor 2, mitogen-activated protein kinase (MAPK), cyclic adenosine 5′-monophosphate-responsive element modulator, peroxisome proliferator-activated receptors (PPARs), O-linked N-acetyl-glucosamine, protein kinase C (PKC), micro ribonucleic acid (microRNA) and exosome pathways[4]. As DCM is now recognized as a cause of substantial morbidity and mortality among patients with diabetes mellitus, affecting 12% of patients with diabetes, various expert groups struggle to identify a suitable biomarker that will help in the recognition and management of DCM[6,7]. The rising burden of DM, estimated to afflict 592 million people by 2035[8], calls attention to this matter even more. Similarly, the prevalence of DM in HF could be over 40%, while in patients with DM, the prevalence of HF ranges from 10% to 22%[9,10]. Unfortunately, so far none of the conducted studies have resulted in the implementation of either conventional cardiac biomarkers or new diagnostic tools in DCM management, yet the current guidelines accentuate the importance of an interdisciplinary team-based approach[11]. Therefore, in this study we sought to address the role of certain biomarkers that have shown potential in either human or animal studies and which could eventually result in mitigating the poor outcomes of DM by participating in the prevention and/or treatment of DCM.

PATHOPHYSIOLOGY OF DIABETIC CARDIOMYOPATHY

So far, most of the underlying pathophysiological mechanisms leading to DCM have

been disclosed[12]. The pathogenesis of DCM is complex and consists of the following systemic and cardiac processes triggered by hyperinsulinemia and increased insulin resistance: impaired coronary microcirculation, dysregulation of the sympathetic nervous system activity and the renin-angiotensin-aldosterone system (RAAS), inappropriate immune response, metabolic disequilibrium of the myocardium and abnormalities of the sub-cellular components. Underlying these pathophysiological events, a role for several proteins and signaling pathways has emerged: adenosine monophosphate-activated protein kinase, PPARs, O-linked N-acetylglucosamine, Sodium-Glucose Cotransporter 2 (SGLT2), PKC, MAPK, NFκB, erythroid 2–related factor 2, microRNA and exosomes[4,13]. Other important mediators implicated in almost every step of DCM development are reactive oxygen species. It is important to point out that these processes are not independent, instead they mutually interact and result in HF. In this review, we highlight some of the above-mentioned pathways relevant for comprehension of the role of biomarkers, as greater details of DCM pathophysiology are beyond the scope of this review. The development of HF in DM is gradual and consists of three distinct phases.

Insulin cell signaling is comprised of two major transduction pathways. The first being phosphatidylinositol 3-kinase-protein kinase B (PI3K-AKT) and the other being MAPK[13]. The PI3K-AKT pathway mainly exerts the metabolic functions of insulin, most important being glucose transporter-4 (GLUT4) cell-surface expression and endothelial nitric oxide (NO) synthase (eNOS) expression. In contrast, the MAPK pathway promotes growth, hypertrophy and remodeling[14]. In an insulin resistant state, these pathways are imbalanced in favor of the MAPK pathway, creating a base for DCM development[15]. Attenuation of the PI3K-AKT and up-regulation of the MAPK pathway are a result of complex interactions between ROS, overfeeding, RAAS activity and many other components which we will discuss further.

Coronary microcirculation seems to be impaired in DM, mediated by multiple pathophysiological processes[16]. Stiffness of small blood vessels is commonly observed among patients with DM, driven by hyperinsulinemia-induced vascular smooth muscle cells differentiation to an osteoblast-like phenotype[17]. In an insulin-resistant state, owing to reduced eNOS levels, NO synthesis is reduced, whereas its degradation is accelerated as a consequence of a heightened state of oxidative stress [18,19]. As it promotes vasodilation *via* guanylyl cyclase activation, a negative balance of NO leads to coronary vasoconstriction[20]. Recent studies suggest a role of the endothelial-to-mesencyhmal transition (EndoMT) in this setting. EndoMT is a mechanistic phenomenon that explains the loss of normal vascular phenotype of endothelial cells, increased cardiac fibroblast content and cardiac fibrosis in the diabetic heart[21]. Importantly, Widyantoro *et al*[22] showed that cardiac fibrosis in the diabetic myocardium is due to stimulation of the EndoMT pathway. It seems that this detrimental cascade which is translated from vasculature onto myocardium could be an important contributor to the onset of HF with preserved ejection fraction (HFpEF) [23].

Altered sympathetic nervous system activity is one of the established hallmarks of DM[24]. The over-expression of β1-adrenergic receptors has been shown to promote myocyte hypertrophy, interstitial fibrosis and myocyte apoptosis[25]. Conversely, sympathetic denervation as a part of cardiac autonomic neuropathy (CAN) is also an important feature of DM. Interestingly, myocardial regions of persistent sympathetic innervation exhibit the greatest deficits of vasodilator reserve[26], thus indicating an association between CAN and impaired myocardial blood flow.

It has been shown that hyperglycemia increases the transcription of angiotensinogen and angiotensin II (At II) production from the local angiotensin converting enzyme, hence increasing the RAAS activity[27]. Accordingly, obesity is also associated with up-regulation of the RAAS[28]. On the other hand, RAAS activity influences insulin signal transduction pathways on multiple levels, which results in an abundance of cardiac and systemic repercussions[29]. By stimulating the creation of ROS *via* nicotinamide adenine dinucleotide phosphate oxidase, as well as by direct phosphorylation of the insulin receptor substrate-1 serine residues, At II inhibits the metabolic PI3K signaling pathway. As eNOs production is mainly dependent on the PI3K pathway[30], At II reduces NO synthesis and thus promotes endothelial dysfunction of myocardial blood vessels[31]. Additionally, aldosterone activation of the mineralocorticoid receptors results in increased ROS production, increased sodium channel expression and activation of serum/glucocorticoid-regulated kinase 1. Altogether, this leads to reduced production of NO and consequently vascular stiffness and impaired cardiac relaxation[32]. Conversely, the MAPK pathway enhance -ment by At II and ROS induces vascular remodeling[33].

Low-grade systemic inflammation and increased polarization towards the pro-inflammatory M1 macrophages and TH1 lymphocytes is fairly common among obese patients and in an insulin-resistant state[15,34,35]. Although regulatory T cells attenuate inflammation in the myocardium, it has been proposed that the secreted pro-inflammatory cytokines, chemokines and growth factors could result in increased cardiac fibrosis and impaired diastolic relaxation[36,37].

An influx of glucose to the myocardial cells is mainly exerted *via* insulin-mediated GLUT4, whereas free fatty acids (FFA) uptake depends on fatty acid translocase (CD36) expression[14,38]. Under physiological circumstances, the heart can use both glucose and FFA as a source of energy. However, in an insulin-resistant state, the expression of GLUT4 diminishes, whereas CD36 expression on plasma membrane is up-regulated. Moreover, elevated levels of intracellular FFA stimulate PPAR-alpha expression, which leads to an increased uptake and oxidation of FFA. Hence, myocardial metabolism shifts from glucose to FFA oxidation, making the myocardium less energy-efficient[39]. As DCM progresses, the expression of genes regulating beta-oxidation of fatty acids is down-regulated, thereby further mitigating the metabolic efficiency of the myocardium[40]. Hyperglycemia leads to the accumulation of the advanced glycation end-products (AGE) *via* non-enzymatic glycation. The AGE induce extracellular matrix cross-linking thus promoting myocardial fibrosis and impaired passive relaxation[13]. Additionally, AGE can stimulate a pro-inflammatory state by binding to the receptors for AGE[41,42]. It should be noted that a relatively novel group of anti-diabetic agents, the SGLT2 inhibitors, have been shown to attenuate hyperglycemia-induced cardiac dysfunction in lipodystrophic mice[43]. In concordance, they exert a cardioprotective effect manifested by improved systolic function, decreased fibrosis and reduced inflammation in At II infusion-induced cardiomyopathy in diabetic mice[44], elucidating the beneficial effects of SGLT2 inhibitors observed in human studies[45]. Mechanisms by which SGLT2 inhibition mitigates DCM and HF in general is an increase in natriuresis, osmotic diuresis, plasma volume contraction, reduction of blood pressure and arterial stiffness and lastly, by providing highly energy-efficient substrates for cardiac metabolism, such as β-hydroxybutyrate[43,45].

Mitochondrial damage is one of the pivotal pathophysiological mechanisms that contribute to DCM. Substrate overflow induces mitochondrial ROS production and impaired oxidative phosphorylation. Consequently, this leads to altered mitochondrial Ca^{2+} handling, which prolongs diastolic relaxation time (diastolic dysfunction) and in later stages leads to cell death[46-48]. Apart from mitochondrial damage, excessive ROS also impair post-translational protein modifications that occur in the endoplasmic reticulum and interfere with insulin signaling pathways. Endoplasmic reticulum stress further stimulates ROS production and favors myocyte apoptosis.

MicroRNAs, small non-coding RNAs, take part in the regulation of mitochondrial function, ROS production, Ca^{2+} handling, apoptosis, autophagy and fibrosis, all of which are regarded as important mechanisms in diabetes induced HF[13]. These microRNAs are transported in exosomes, recently recognized extracellular vesicles involved in cell-to-cell communication[49].

The development of DCM can be divided in three distinct phases (Figure 1)[13]. In the initial phase, there are no obvious changes in the myocardium tissue and systolic function is preserved[50]. However, using echocardiography and magnetic resonance imaging (MRI) in rodents, authors observed subtle anomalies that indicate impaired diastolic relaxation. MRI findings that pointed to the impaired diastolic relaxation were slow initial and peak filling rates, whereas abnormal myocardial performance index, long period of isovolumic relaxation and impaired septal annular wall motion were the observed echocardiographic diastolic parameters[51,52,15]. In humans, early DCM is characterized by increased cardiac stiffness and impaired cardiac relaxation with consequent reduction in early diastolic filling and an increase in atrial filling [50, 53]. In addition, another hallmark is a decrease in the myocardial blood-flow reserve that can be detected by various imaging techniques[54]. Needless to say, the whole initial phase is completely asymptomatic. As underlying pathophysiological mechanisms continue to exhibit their deleterious cellular effects on cardiac tissue, DCM becomes more and more evident. In the advanced phase, as myocardium becomes hypertrophic and increasingly permeated with fibrous tissue, left ventricular mass and wall thickness both increase and hence, diastolic dysfunction becomes clinically apparent. In this phase, patients may notice first symptoms which correspond to the symptoms observed in HFpEF, the most prominent being exercise intolerance[55,56]. In the late phase of DCM development, except for diastolic dysfunction, further progression of cardiac remodeling finally results in mitigation of systolic function and consequent HF with reduced ejection fraction[12]. It is important to note

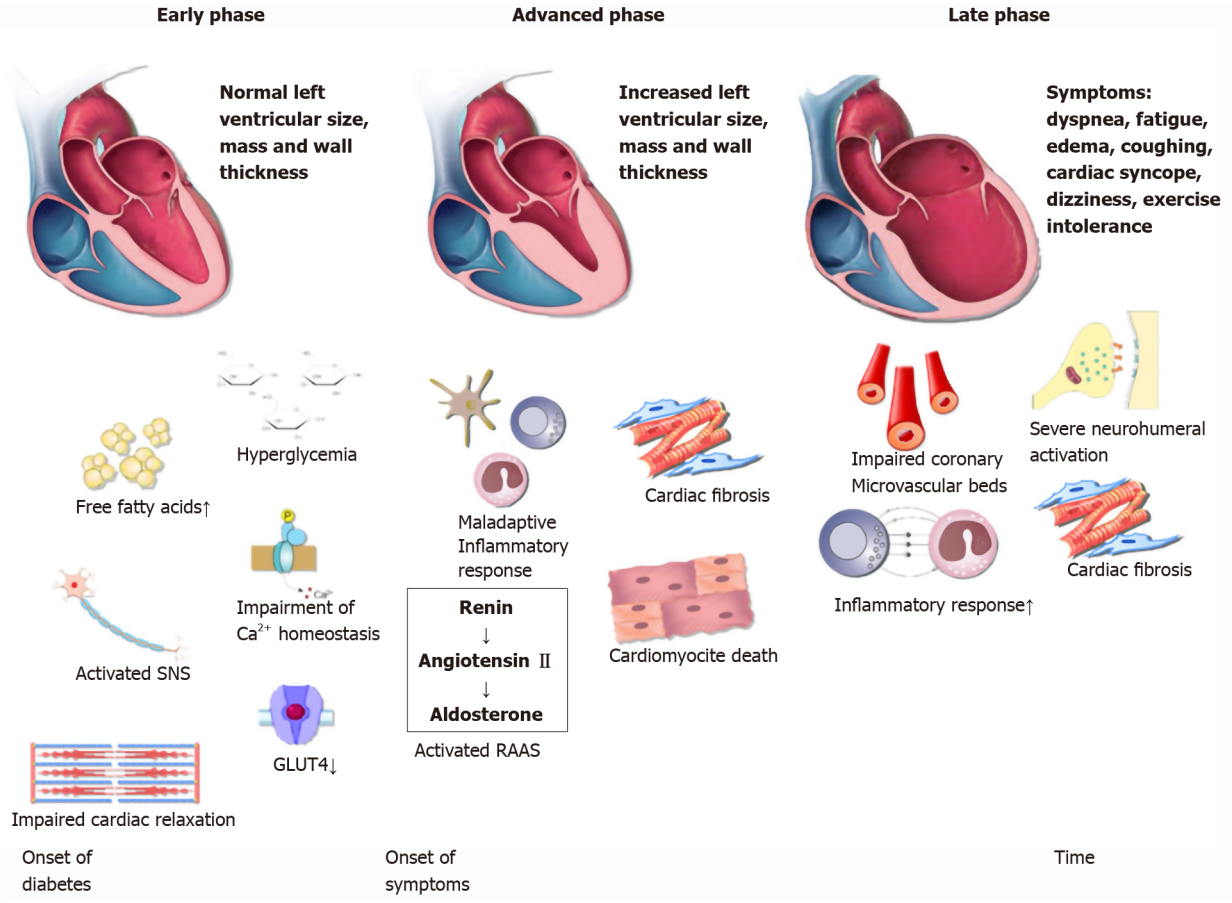

Figure 1 Schematic representation of the diabetic cardiomyopathy phases. Each background color corresponds to its respective phase: green (early phase), yellow (advanced phase), red (late phase). The red line represents the incremental nature of symptom severity. SNS: Sympathetic nervous system; GLUT4: Glucose transporter type 4; RAAS: Renin-Angiotensin-Aldosterone System.

that DCM staging still remains theoretical, since it is difficult to reach a definite diagnosis of DCM. However, we believe that staging is useful because it highlights the importance of timely DCM diagnosis.

BIOMARKERS IN DIABETIC CARDIOMYOPATHY

Role of traditional cardiac biomarkers in the management of diabetic cardiomyopathy

Established cardiac biomarkers used for detection in patients with HF have failed to timely recognize DCM. Brain natriuretic peptide (BNP) correlation with HF is blunted owing to the association between BNP and insulin resistance[57]. In contrast, N-terminal pro-BNP and ANP have been able to predict HF in experimental DCM rat models[58,59]. Furthermore, both natriuretic peptides successfully demonstrated diastolic dysfunction in diabetics and in conjunction with 2D echocardiography [60, 61]. However, their value as a biomarker was limited to symptomatic patients, those with pseudo-normalized mitral flow pattern and those with a restrictive filling pattern [60]. There was no correlation of these natriuretic peptides with diastolic dysfunction among asymptomatic patients and those with relaxation abnormalities. Additional studies also demonstrated a lack of correlation among asymptomatic patients with diastolic dysfunction and overall poor correlation with most of the echocardiography parameters[62,63]. In conclusion, the utility of natriuretic peptides in pre-clinical DCM detection is limited; however, BNP seems to be an independent predictor of poor outcomes in this cardiomyopathy[64-66].

Another family of entrenched cardiac markers are the troponins, a set of proteins that control the calcium-mediated interaction between actin and myosin. This multiprotein complex consists of troponin C which binds calcium, troponin T (TnT) which binds to tropomyosin and troponin I (TnI) which prevents actin-myosin

interaction[67]. Cardiac troponin I and T are commonly used in routine clinical practice due to their high sensitivity and specificity for the detection of myocardial injury[68]. Both human and animal studies suggest that TnI and TnT are constitutively phosphorylated in diabetes *via* PKC, leading to depressed myofilament function and Ca^{2+} responsiveness[69,70]. Of note, losartan, an At II receptor blocker seems to abrogate TnI phosphorylation[71]. Although it is well-known that TnT and TnI are elevated among patients with diabetes, especially among those with concomitant coronary artery disease, to our knowledge no studies have investigated the difference in troponin plasma levels between diabetics with DCM and diabetics without DCM[72,73]. Taken together, established laboratory biomarkers measuring myocardial injury and mechanical hemodynamic overload of the ventricles are not specific markers of DCM.

Novel biomarkers of diabetic cardiomyopathy

Cardiotrophin-1 (CT-1), a member of the glycoprotein 130 family, is a potent inducer of cardiomyocyte hypertrophy *in vitro*[74]. CT-1 secretion is stimulated by various triggers: mechanical stretch of cardiomyocytes, hypoxic stress, ROS, At II, aldosterone, urocortin, glucose, insulin and fibroblast growth factor-2[75-82]. Triggered by any of the above-mentioned, CT-1 modulates myocardial contractility, fibrosis and cardiac conduction *via* activation of the JAK/STAT and MAPK pathways (Figure 2)[83,84]. Apart from its effects on heart remodeling, CT-1 also takes part in cardiac glucose metabolism by increasing insulin-stimulated glucose uptake[85,86]. In line with this, plasma CT-1 levels are positively correlated with basal glycemia and left ventricular hypertrophy[87]. Other studies showed elevated plasma levels of CT-1 in recently diagnosed diabetics and neonates exposed to maternal diabetes[88], but interestingly, reduced levels in obese non-diabetics[89,90]. Moreover, low CT-1 plasma levels seem to be associated with decreased risk of both metabolic syndrome and DM type 2 in obese subjects[91]. Although CT-1 is to a great extent implicated in DCM, there are two major setbacks that prevent CT-1 implementation in the DCM diagnostic algorithm [92]. Firstly, CT-1 is also expressed by various tissues such as liver, lung, kidney and skeletal muscle[93]. Secondly, CT-1 plasma level alterations are also associated with other types of cardiomyopathies, including ischemic, making it less specific[84].

Insulin-like growth factor binding protein 7 (IGFBP7) is a part of the IGFBP superfamily of homogenous proteins which regulate the IGF signaling pathway by binding with insulin and IGFs[94]. Unlike IGFBP 1-6, IGFBP7 has low binding affinity to IGF but high affinity to insulin[95]. Owing to its high binding affinity to insulin, IGFB7 may interfere with the biological response of insulin, subsequently inducing insulin resistance and is involved in the development of diabetes, as shown by multiple studies (Figure 2)[96,97]. Apart from its role in insulin signaling, IGFBP7 is associated with multiple processes including fibrogenesis and tumor development [98,99]. IGFBP7 has also been implicated in HF where it serves as a novel prognostic biomarker for heart failure with reduced ejection fraction and shows a significant correlation with the presence and severity of the echocardiographic parameters of abnormal diastolic function[100]. In a recent study, the potential of IGFBP7 in improving the diagnosis of acute HF has been highlighted[101]. The most important evidence of IGFBP7 utility in the setting of DCM was provided by Shaver *et al*[102] who tested the potential of various serum biomarkers in a West Virginian population. The authors compared plasma levels between controls and diabetics (DM group), but more importantly, between diabetics with diastolic dysfunction (DM, DD+ group) and diabetics without diastolic dysfunction (DM, DD- group). IGFBP7 plasma levels were significantly higher in the DM, DD+ group in comparison to the DM, DD- group. Given their role in insulin resistance, fibrogenesis, HF development and the results presented by Shaver *et al*[102], we argue that further research of IGFBP7 in this manner is valuable as it could be a candidate for early detection of DCM.

Another important finding by Shaver *et al*[102] is in regards to transforming growth factor-β (TGF-β), a ubiquitous fibrogenic cytokine that promotes extracellular matrix accumulation[103]. As a result of increased ROS production, TGF-β is up-regulated in patients with diabetes[104]. Additionally, TGF-β correlates with the degree of cardiac fibrosis[105]. Of note, although most of the TGF-β-induced cardiac fibrosis is exerted by modulating the fibroblast phenotype and function[106], an additional mechanism that may contribute to fibrosis is TGF-β-mediated induction of EndoMT[107,108], a deleterious process implicated in HFpEF pathophysiology[23]. Shaver *et al*[102] reported higher plasma levels of TGF-β in patients with both DM and DD in comparison to the other two groups, respectively. Therefore, TGF-β could serve as a marker in DCM management. This is in line with previous studies conducted on this topic. By using FT23, an orally active anti-fibrotic compound, Tan *et al*[109] success-

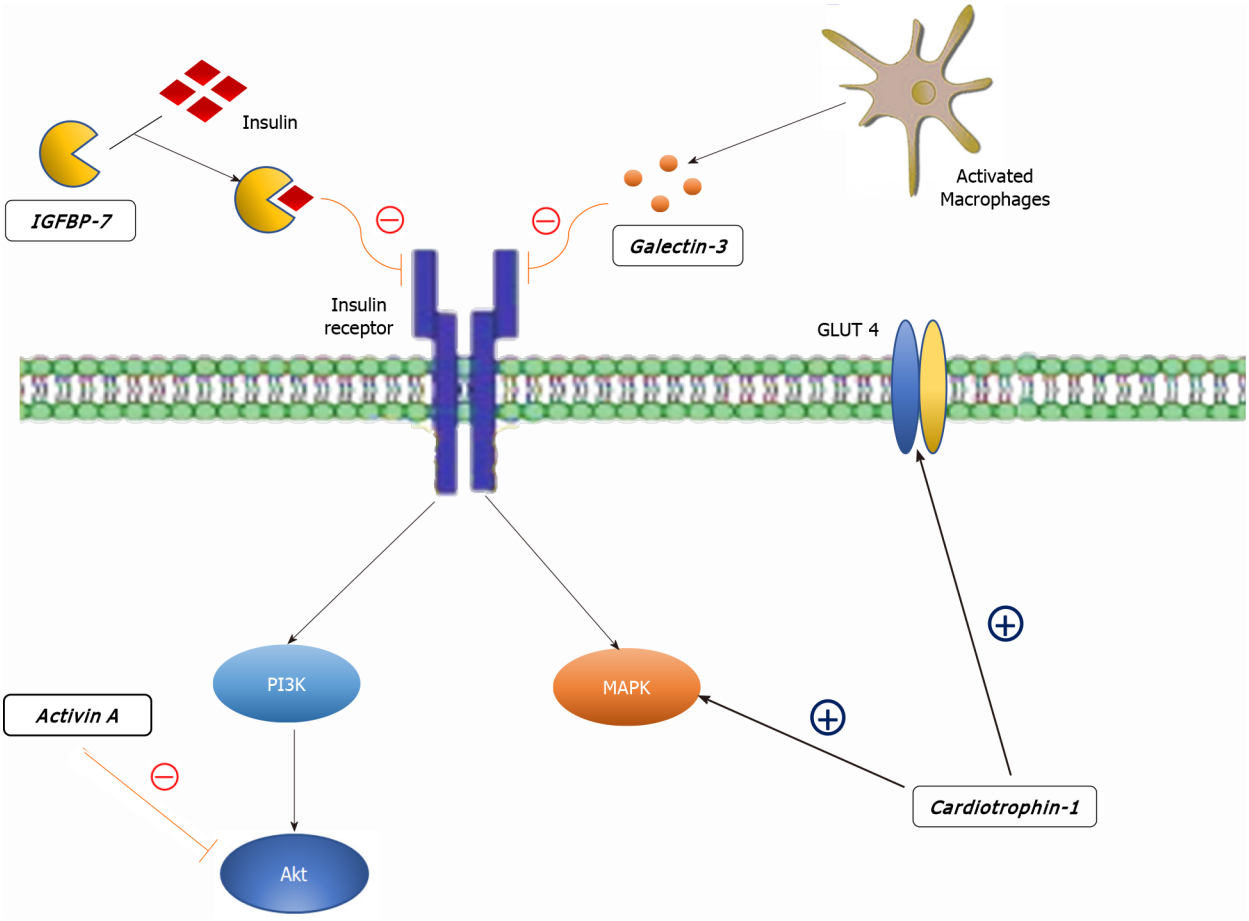

Figure 2 Molecular targets of the diabetic cardiomyopathy biomarkers in cardiomyoctes. MAPK: Mitogen-activated protein kinase; PI3K: Phosphatidylinositol 3-kinase-protein kinase B; IGFBP7: Insulin-like growth factor binding protein 7; GLUT4: Glucose transporter type 4.

fully demonstrated the TGF-β-mediated attenuation of diastolic dysfunction in an experimental model of DCM. In line with the latter, Smad3, a signaling pathway by which TGF-β exerts a part of its pro-fibrotic features, has been shown to mediate diabetic cardiac hypertrophy, fibrosis, and diastolic dysfunction[110,111].

Activin A, a protein secreted by epicardial adipose tissue (EAT) is another member of the TGF-β superfamily that seems to be involved in the development of DCM[112]. Greulich *et al*[113] demonstrated that excessive activation of Activin A-signaling results in contractile dysfunction and insulin resistance in high fat diet fed guinea pigs. The underlying mechanism seems to be inhibition of insulin-mediated phosphorylation of rrAkt, a key regulator of myocardial glucose uptake (Figure 2)[114]. In addition, authors also observed decreased calcium ATPase-2a expression and sarcomere shortening. By cultivating rat cardiomyocytes with EAT byoptates derived from diabetics, Blumensatt *et al*[115] highlighted the role of microRNA in Activin A-induced insulin inhibition and led to further disclosure of DCM pathophysiology. Finally, the potential of Activin A as a biomarker in diabetes has been exploited by Chen *et al*[116]. These authors reported an association between Activin A plasma levels and both impaired myocardial glucose metabolism and left ventricular remodeling in patients with uncomplicated type II diabetes[116]. In contrast to diastolic dysfunction and HF, Activin A is not elevated in uncomplicated DM, which could be beneficial for its utility as a biomarker. However, we doubt that Activin A will find clinical implications in this manner, as its plasma levels are affected by metformin, a ubiquitous diabetes medication, and the secretion of Activin A is not limited to EAT but it is also expressed by many other cells[116-121].

Considering the importance of ROS overproduction in DCM pathophysiology and the well-known ROS-induced inflammatory response, multiple authors have tested the potential of inflammatory markers in this setting. A recent study on core gene biomarkers in patients with DCM addressed the vital role of interleukin-6 in DCM pathophysiology[122]. Furthermore, Shaver *et al*[102] found that both interleukin-6 and tumor necrosis factor-alpha are more increased in patients with both DM and DD in contrast to patients with DM exclusively. Nevertheless, owing to the low specificity

of the two, it seems that growth differentiation factor-15 (GDF-15), another inflammatory marker, has a much better chance of being implemented in DCM diagnosis [123]. GDF-15, another member of the TGF-β superfamily is produced in response to oxidative stress and inflammation by multiple cell types, including macrophages, adipocytes, and cardiovascular cells[123]. Elevated plasma levels of GDF-15 seem to be associated with increased risk in fatal and non-fatal cardiovascular events of community-dwelling subjects and patients with cardiovascular disease, as shown by multiple studies[125-127]. Interestingly, in these studies GDF-15 levels were higher among patients with established DM type 2. Additionally, several studies addressed the contribution of GDF-15 in diastolic dysfunction[128,129]. As demon-strated by Dominguez-Rodriguez *et al*[130], elevated levels of GDF-15 can predict DCM development in the absence of other risk factors, such as age, smoking, hypertension and known cardiovascular disease. Importantly, multiple authors have shown that GDF-15 expression in various tissues is higher in pre-diabetes and DM type 2 patients in comparison to individuals without the mentioned metabolic disorders, making GDF-15 a promising biomarker for identification of DCM and its repercussions among diabetics[131,132]. Notably, a new class of GFRAL (high affinity binding receptor for GDF-15)/RET (receptor tyrosine kinase)-based drugs for the treatment of obesity and metabolic syndrome could improve cardiovascular risk in individuals with metabolic diseases by mediating the endogenous effects of GDF-15[133].

Galectin-3 is a lectin family protein that has been associated with fibrosis and inflammation in cardiac, kidney and liver diseases[134,135]. Galectin-3 levels correlate with accumulation of AGE, oxidative stress products and pro-apoptotic pathways which directly promote endothelial dysfunction[136,137]. Perhaps the most important role of galectin-3 is its role in HF, where galectin-3 is an important mediator by which multiple molecules, such as At II and aldosterone, exert their pro-fibrotic activity and where it is able to promote oxidative stress with well-known repercussions[138-143]. The first evidence to support these findings were provided by Sharma *et al*[144] in a study which demonstrated that galectin-3 was the strongest differentially regulated gene associated with HF. Subsequently, a number of authors produced abundant evidence that successfully associated galectin-3 with HF in both animal models and in human studies, leading to the Food and Drug Administration approval of galectin-3 as a novel biomarker for predicting cardiovascular adverse events in 2010[145-149]. It is important to note that inhibition of galectin-3 could be an important target molecule in the HF therapeutic approach, based on its potential to undermine cardiac fibrosis and mitigate poor outcomes of HF. Multiple studies have highlighted the link between DM type 2 and galectin-3. The Dallas Heart Study associated galectin-3 with diabetes prevalence and incidence even after adjustment for conventional metabolic and cardiovascular risk factors[150]. Furthermore, in young obese patients without known cardiovascular disease, galectin-3 is associated with the presence of left ventricular diastolic dysfunction and elevated pulmonary artery systolic pressure, indicating its possible role in screening for preclinical metabolic heart disease[151]. On the other hand, in patients with HF, galectin-3 plasma levels were higher among those with impaired glucose metabolism (Figure 2)[152]. Finally, the possible role of galectin-3 in the DCM diagnostic approach was evaluated in a recent study by Flores-Ramírez *et al* [153]. The study showed that galectin-3 is elevated in diabetic patients with mild depressed ejection fraction and is associated with a diminished global longitudinal strain, an easy and reproducible echocardiographic tool in the evaluation and follow-up of DCM[154].

The soluble form of suppression of tumorigenicity 2 (sST2) is a interleukin-33 (IL-33) decoy receptor that tones down the Th2 inflammatory response *via* the IL-33/ST2/sST2 axis (Figure 3)[155]. Consequently, the protective effects of IL-33 in atherosclerosis and cardiac remodeling are mitigated, as this axis is an important component of the autocrine/paracrine mechanism that prevents tissue injury[156, 157]. Increased plasma concentrations of sST2 are not specific for a single disorder in humans which undermines its value as a biomarker[158]. However, increased plasma levels of sST2 have been linked to a worse prognosis in numerous diseases, the most important being HF[159-162]. In line with this, sST2 is now included in the 2017 ACCF/AHA guidelines for additive risk stratification of patients with acute and chronic HF[163]. In the case of diabetes, Fousteris *et al*[164] demonstrated higher plasma concentrations of sST2 among patients with DM type 2 in comparison to healthy controls. More importantly, authors observed even higher levels of sST2 in patients with both DM type 2 and grade I left ventricular diastolic dysfunction, an early finding in DCM[165]. The presented data suggest a possible association between sST2 and the early stages of DCM; however, a larger body of evidence is needed to support these findings.

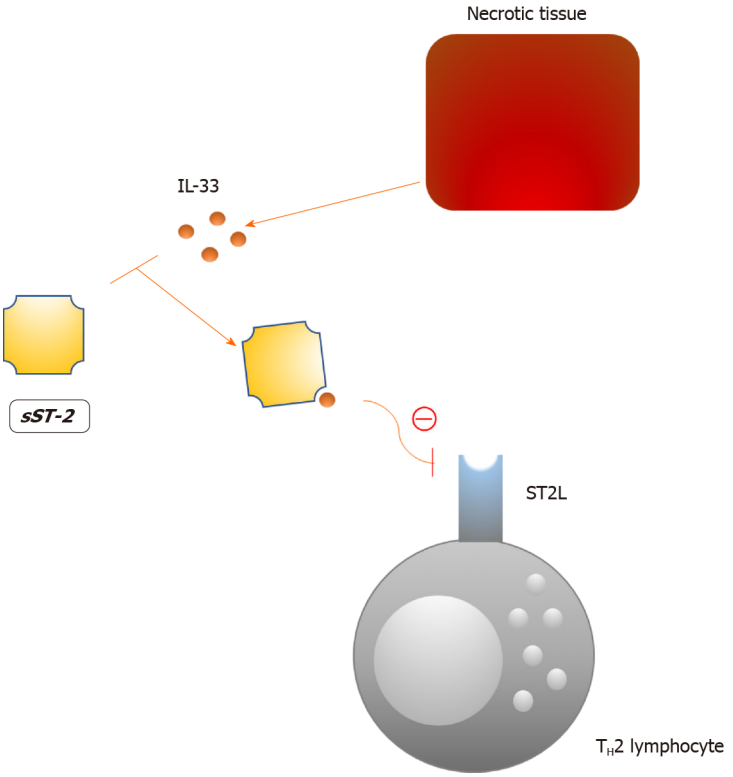

Figure 3 Molecular target of the soluble form of suppression of tumorigenicity 2. sST2: Soluble form of suppression of tumorigenicity 2; IL-33: Interleukin-33; ST2L: Suppression of tumorigenicity 2 ligand; T_H2: T helper lymphocyte type 2.

Long noncoding RNAs (lncRNAs) are a diverse subgroup of noncoding RNAs comprised of sequences longer than 200 nucleotides that act as epigenetic regulators of gene expression[166]. There is a large body of evidence indicating that lncRNAs are implicated in cardiac development, function and diseases[167,168]. Recent studies suggest that circulating lncRNAs could serve as diagnostic and prognostic biomarkers of cardiac remodeling and survival in cardiovascular diseases[169,170]. Both *in vitro* and *in vivo* studies showed that lncRNAs are involved in the pathophysiology of diabetes and its complications[171-173]. The most important study that addressed the potential of multiple lncRNAs as early DCM biomarkers was conducted by de Gonzalo-Calvo *et al*[174]. These authors compared a panel of lncRNAs that are directly involved in either diabetic conditions or cardiovascular disease and attempted to determine their relationship with MRI indices of cardiac dimensions and function. Long intergenic non-coding RNA predicting cardiac remodeling (LIPCAR) was inversely associated with E/A peak flow, an established indicator of diastolic dysfunction. In addition, LIPCAR serum levels positively correlated with grade I diastolic dysfunction. However, although LIPCAR was also correlated with waist circumference, plasma fasting insulin, subcutaneous fat volume and HDL-C, which could seemingly undermine LIPCAR value as a specific biomarker of cardiac impairment, the observed correlation with cardiac dysfunction was independent of the aforementioned. On the other hand, smooth muscle and endothelial cell-enriched migration/differentiation-associated long noncoding RNA (SENCR) and myocardial infarction-associated transcript (MIAT) lncRNAs serum levels were both associated with left ventricular mass to volume ratio, a marker of cardiac remodeling, even after adjustment for possible confounding factors. Notably, the highest left ventricular mass to volume ratios were observed in patients with the highest MIAT and SENCR expression. It is also important to point out that neither SENCR nor MIAT levels correlated with other clinical, biochemical, or metabolic parameters, which supports the hypothesized utility of these lncRNAs as biomarkers of left ventricular remodeling.

MicroRNAs are small noncoding RNA molecules which regulate gene expression by post-transcriptional mechanisms[175]. These molecules control around 30% of all protein-coding genes of the mammalian genome[176]. Additionally, microRNAs are also paracrine mediators of cell-to-cell communication transported *via* exosomes, a mechanism which has lately become an emerging research field for understanding the

development of cardiac pathology[177]. The release of circulating exosomes filled with microRNA in the bloodstream from cardiomyocytes, driven by oxidative stress or hypoxia/reoxygenation, as well as stable microRNA-protein complex transport, makes microRNA an attractive target for analytical studies[178-182]. Recent pre-clinical level studies identified several distinct microRNAs which have been involved in DCM pathophysiology. Among many, we highlighted those we thought most suitable for DCM diagnosis based on their pathophysiologic role in DCM: microRNA-223 which regulates Glut4 receptor expression and cardiomyocyte glucose uptake and microRNA-133a which is implicated in cardiac hypertrophy and myocardial matrix remodeling[183-185]. Despite their potential, there are currently no ongoing clinical trials regarding the role of microRNAs in this manner. Perhaps the biggest setback in using microRNAs as markers is discordance between human and animal serum microRNAs associated with DCM[186]. The only exceptions are microRNA-34a, a regulator of high glucose-induced apoptosis and microRNA-30d, a molecule involved in the process of cardiomyocyte pyroptosis[187,188].

CONCLUSION

Despite substantial efforts to establish appropriate diagnostic biomarkers of DCM, this entity is not even diagnosed among clinicians, mainly due to the absence of agreement among experts[4]. Hence, new strategies must be applied in order to ameliorate poor outcomes of diabetes-related HF. In an ideal setting, DCM would be recognized in the early asymptomatic phase, before irreversible myocardial damage occurs. Different imaging approaches such as Phase-MRI, Speckle tracking echocardiography (STE) and nuclear imaging have been successful in the recognition of early metabolic myocardial changes in both animal and human studies[189-194]. However, most of these are limited by price and availability, whereas STE, although promising, can have reduced accuracy in irregular ventricular remodeling and wall thinning[6]. Importantly, global longitudinal strain, an echocardiographic measurement, seems to be more impaired in DM *vs* healthy controls whereas among diabetics, it is more impaired in patients with albuminuria in comparison to patients without it[195]. In addition, patients with uncomplicated DM type II show a similar time-dependent pattern of global longitudinal strain change, altogether indicating subclinical systolic dysfunction in patients with diabetes that is associated with duration and extent of the disease[196]. Of the aforementioned biomarkers, we believe that lncRNA, sST2 and galectin-3 will be the most beneficial for DCM detection, as their plasma/serum levels accurately correlate with the early stages of DCM.

In addition, there are several molecules which are rarely debated in this manner and which we find valuable for further research based on their functional properties. Catestatin, a pleiotropic cardioprotective peptide that counterbalances the negative effects of the sympathetic nervous system, is implicated in both the metabolic syndrome and HF[197]. Specifically, alongside sST2, our recent study suggested that catestatin plasma levels reflect myocardial fibrosis and sympathetic overactivity during the acute worsening of HF[198]. With regard to diabetes, catestatin has been shown to increase glucose uptake and up-regulate GLUT4 plasma expression in rat cardiomyocytes[199], as well as improve insulin sensitivity in mice with diet-induced obesity[200].

To sum up, further research is needed to improve DCM approach strategies. The combination of relatively inexpensive and accurate STE with some of the highlighted biomarkers seems promising (Table 1); however, well-designed studies with long-term follow-up and validation are obligatory for implementation in everyday clinical practice. With the exception of "What to test?", rather more important questions are "When and whom to test". Given that DCM affects around 12% of diabetics, we need a predictive scoring system to establish that a patient is at risk of DCM development, as they all are. Thus, screening methods should be applied for all newly-diagnosed type 2 DM patients. In DM type 1, due to the discrepancy in certain pathophysiological aspects in respect to DM type 2, further research is needed to reach proper conclusions [201,202]. With regard to "When to test?", as DCM progression deteriorates heart function stepwise and as new therapeutic strategies that specifically target early phase mechanisms emerge, it will be vital to detect DCM as soon as possible. Finally, we argue that an effort must be made to create an easy and reproducible algorithm which will, by using a combination of STE and biomarkers, direct affected patients to confirmation tests such as Phase-MRI. Consequently, in patients with validated DCM, new specific therapies that target early phase mechanisms could be applied. This type

Table 1 Promising novel biomarkers in diagnostic approach to diabetic cardiomyopathy

Biomarker	Pathophysiological pathway	Supporting evidence
LncRNA (LIPCAR, MIAT, SENCR)	Epigenetic regulation of multiple genes involved in diabetes and cardiac dysfunction	Liu *et al*[171]; Yan *et al*[172]; Carter *et al*[173]; de Gonzalo-Calvo *et al*[174]
sST-2	IL-33 decoy receptor that tones down Th2 inflammatory response *via* the IL-33/ST2/sST2 axis	Fousteris *et al*[164]; Kiencke *et al*[165]
TGF-β	The main pro-fibrotic factor in heart failure: it modulates the fibroblast phenotype and function and mediates induction of EndoMT	Shaver *et al*[102]; Iglesias-De La Cruz *et al*[104]; Asbun *et al*[105]
Galectin-3	Mediator by which multiple molecules (*e.g.* angiotensin II and aldosterone) exert their pro-fibrotic activity and promote oxidative stress	Ho *et al*[146]; Ueland *et al*[147]; Sharma *et al*[148]
GDF-15	Regulator of inflammatory pathways involved in regulation of apoptosis, cell repair and cell growth	Berezin[123]; Dominguez-Rodriguez *et al*[130]

RNA: Ribonucleic acid; LncRNA: Long noncoding ribonucleic acid; LIPCAR: Long intergenic non-coding ribonucleic acid predicting cardiac remodeling; MIAT: Myocardial infarction-associated transcript; SENCR: Smooth muscle and endothelial cell-enriched migration/differentiation-associated long noncoding ribonucleic acid; sST2: Soluble form of suppression of tumorigenicity 2; GDF-15: Growth differentiation factor-15; TGF-β: Transforming growth factor-β; EndoMT: Endothelial-mesenchymal transition.

of approach is needed to stratify patients because most of the new therapies will be very costly.

ACKNOWLEDGEMENTS

The figures were kindly provided by Zrinka Miocic, M.Arch.

REFERENCES

1 **Rubler S**, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol* 1972; **30**: 595-602 [PMID: 4263660 DOI: 10.1016/0002-9149(72)90595-4]

2 **Kannel WB**, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol* 1974; **34**: 29-34 [PMID: 4835750 DOI: 10.1016/0002-9149(74)90089-7]

3 **Yancy CW**, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2013; **62**: e147-e239 [PMID: 23747642 DOI: 10.1016/j.jacc.2013.05.019]

4 **Lee MMY**, McMurray JJV, Lorenzo-Almorós A, Kristensen SL, Sattar N, Jhund PS, Petrie MC. Diabetic cardiomyopathy. *Heart* 2019; **105**: 337-345 [PMID: 30337334 DOI: 10.1136/heartjnl-2016-310342]

5 **Ponikowski P**, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; ESC Scientific Document Group. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016; **37**: 2129-2200 [PMID: 27206819 DOI: 10.1093/eurheartj/ehw128]

6 **Berezin AE**, Berezin AA. Circulating Cardiac Biomarkers in Diabetes Mellitus: A New Dawn for Risk Stratification-A Narrative Review. *Diabetes Ther* 2020; **11**: 1271-1291 [PMID: 32430864 DOI: 10.1007/s13300-020-00835-9]

7 **Lorenzo-Almorós A**, Tuñón J, Orejas M, Cortés M, Egido J, Lorenzo Ó. Diagnostic approaches for diabetic cardiomyopathy. *Cardiovasc Diabetol* 2017; **16**: 28 [PMID: 28231848 DOI: 10.1186/s12933-017-0506-x]

8 **Tao Z**, Shi A, Zhao J. Epidemiological Perspectives of Diabetes. *Cell Biochem Biophys* 2015; **73**:

181-185 [PMID: 25711186 DOI: 10.1007/s12013-015-0598-4]

9 **Greenberg BH**, Abraham WT, Albert NM, Chiswell K, Clare R, Stough WG, Gheorghiade M, O'Connor CM, Sun JL, Yancy CW, Young JB, Fonarow GC. Influence of diabetes on characteristics and outcomes in patients hospitalized with heart failure: a report from the Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE-HF). *Am Heart J* 2007; **154**: 277.e1-277. e8 [PMID: 17643576 DOI: 10.1016/j.ahj.2007.05.001]

10 **Nichols GA**, Gullion CM, Koro CE, Ephross SA, Brown JB. The incidence of congestive heart failure in type 2 diabetes: an update. *Diabetes Care* 2004; **27**: 1879-1884 [PMID: 15277411 DOI: 10.2337/diacare.27.8.1879]

11 **Dunlay SM**, Givertz MM, Aguilar D, Allen LA, Chan M, Desai AS, Deswal A, Dickson VV, Kosiborod MN, Lekavich CL, McCoy RG, Mentz RJ, Piña IL; American Heart Association Heart Failure and Transplantation Committee of the Council on Clinical Cardiology; Council on Cardiovascular and Stroke Nursing; and the Heart Failure Society of America. Type 2 Diabetes Mellitus and Heart Failure: A Scientific Statement From the American Heart Association and the Heart Failure Society of America: This statement does not represent an update of the 2017 ACC/AHA/HFSA heart failure guideline update. *Circulation* 2019; **140**: e294-e324 [PMID: 31167558 DOI: 10.1161/CIR.0000000000000691]

12 **Jia G**, Hill MA, Sowers JR. Diabetic Cardiomyopathy: An Update of Mechanisms Contributing to This Clinical Entity. *Circ Res* 2018; **122**: 624-638 [PMID: 29449364 DOI: 10.1161/CIRCRESAHA.117.311586]

13 **Jia G**, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol* 2016; **12**: 144-153 [PMID: 26678809 DOI: 10.1038/nrendo.2015.216]

14 **Dhalla NS**, Pierce GN, Innes IR, Beamish RE. Pathogenesis of cardiac dysfunction in diabetes mellitus. *Can J Cardiol* 1985; **1**: 263-281 [PMID: 3850773]

15 **Jia G**, Habibi J, Bostick BP, Ma L, DeMarco VG, Aroor AR, Hayden MR, Whaley-Connell AT, Sowers JR. Uric acid promotes left ventricular diastolic dysfunction in mice fed a Western diet. *Hypertension* 2015; **65**: 531-539 [PMID: 25489061 DOI: 10.1161/HYPERTENSIONAHA.114.04737]

16 **Factor SM**, Minase T, Cho S, Fein F, Capasso JM, Sonnenblick EH. Coronary microvascular abnormalities in the hypertensive-diabetic rat. A primary cause of cardiomyopathy? *Am J Pathol* 1984; **116**: 9-20 [PMID: 6742110]

17 **Olesen P**, Nguyen K, Wogensen L, Ledet T, Rasmussen LM. Calcification of human vascular smooth muscle cells: associations with osteoprotegerin expression and acceleration by high-dose insulin. *Am J Physiol Heart Circ Physiol* 2007; **292**: H1058-H1064 [PMID: 17056676 DOI: 10.1152/ajpheart.00047.2006]

18 **Sharma A**, Sellers S, Stefanovic N, Leung C, Tan SM, Huet O, Granville DJ, Cooper ME, de Haan JB, Bernatchez P. Direct Endothelial Nitric Oxide Synthase Activation Provides Atheroprotection in Diabetes-Accelerated Atherosclerosis. *Diabetes* 2015; **64**: 3937-3950 [PMID: 26116699 DOI: 10.2337/db15-0472]

19 **Zhou X**, Ma L, Habibi J, Whaley-Connell A, Hayden MR, Tilmon RD, Brown AN, Kim JA, Demarco VG, Sowers JR. Nebivolol improves diastolic dysfunction and myocardial remodeling through reductions in oxidative stress in the Zucker obese rat. *Hypertension* 2010; **55**: 880-888 [PMID: 20176997 DOI: 10.1161/HYPERTENSIONAHA.109.145136]

20 **Hayden MR**, Habibi J, Joginpally T, Karuparthi PR, Sowers JR. Ultrastructure Study of Transgenic Ren2 Rat Aorta - Part 1: Endothelium and Intima. *Cardiorenal Med* 2012; **2**: 66-82 [PMID: 22493605 DOI: 10.1159/000335565]

21 **Li Q**, Yao Y, Shi S, Zhou M, Zhou Y, Wang M, Chiu JJ, Huang Z, Zhang W, Liu M, Wang Q, Tu X. Inhibition of miR-21 alleviated cardiac perivascular fibrosis *via* repressing EndMT in T1DM. *J Cell Mol Med* 2020; **24**: 910-920 [PMID: 31680453 DOI: 10.1111/jcmm.14800]

22 **Widyantoro B**, Emoto N, Nakayama K, Anggrahini DW, Adiarto S, Iwasa N, Yagi K, Miyagawa K, Rikitake Y, Suzuki T, Kisanuki YY, Yanagisawa M, Hirata K. Endothelial cell-derived endothelin-1 promotes cardiac fibrosis in diabetic hearts through stimulation of endothelial-to-mesenchymal transition. *Circulation* 2010; **121**: 2407-2418 [PMID: 20497976 DOI: 10.1161/CIRCULATIONAHA.110.938217]

23 **Elgendy IY**, Pepine CJ. Heart Failure With Preserved Ejection Fraction: Is Ischemia Due to Coronary Microvascular Dysfunction a Mechanistic Factor? *Am J Med* 2019; **132**: 692-697 [PMID: 30684452 DOI: 10.1016/j.amjmed.2018.12.038]

24 **Thackeray JT**, Beanlands RS, Dasilva JN. Altered sympathetic nervous system signaling in the diabetic heart: emerging targets for molecular imaging. *Am J Nucl Med Mol Imaging* 2012; **2**: 314-334 [PMID: 23133819]

25 **Bisognano JD**, Weinberger HD, Bohlmeyer TJ, Pende A, Raynolds MV, Sastravaha A, Roden R, Asano K, Blaxall BC, Wu SC, Communal C, Singh K, Colucci W, Bristow MR, Port DJ. Myocardial-directed overexpression of the human beta(1)-adrenergic receptor in transgenic mice. *J Mol Cell Cardiol* 2000; **32**: 817-830 [PMID: 10775486 DOI: 10.1006/jmcc.2000.1123]

26 **Stevens MJ**, Dayanikli F, Raffel DM, Allman KC, Sandford T, Feldman EL, Wieland DM, Corbett J, Schwaiger M. Scintigraphic assessment of regionalized defects in myocardial sympathetic innervation and blood flow regulation in diabetic patients with autonomic neuropathy. *J Am Coll Cardiol* 1998; **31**: 1575-1584 [PMID: 9626837 DOI: 10.1016/s0735-1097(98)00128-4]

27 **Lee MH**, Song HK, Ko GJ, Kang YS, Han SY, Han KH, Kim HK, Han JY, Cha DR. Angiotensin receptor blockers improve insulin resistance in type 2 diabetic rats by modulating adipose tissue. *Kidney Int* 2008; **74**: 890-900 [PMID: 18596725 DOI: 10.1038/ki.2008.313]

28 **Shoelson SE**, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; **116**: 1793-1801 [PMID: 16823477 DOI: 10.1172/JCI29069]

29 **Zhou MS**, Schulman IH, Zeng Q. Link between the renin-angiotensin system and insulin resistance: implications for cardiovascular disease. *Vasc Med* 2012; **17**: 330-341 [PMID: 22814999 DOI: 10.1177/1358863X12450094]

30 **Andreozzi F**, Laratta E, Sciacqua A, Perticone F, Sesti G. Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser616 in human umbilical vein endothelial cells. *Circ Res* 2004; **94**: 1211-1218 [PMID: 15044323 DOI: 10.1161/01.RES.0000126501.34994.96]

31 **Wei Y**, Sowers JR, Nistala R, Gong H, Uptergrove GM, Clark SE, Morris EM, Szary N, Manrique C, Stump CS. Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. *J Biol Chem* 2006; **281**: 35137-35146 [PMID: 16982630 DOI: 10.1074/jbc.M601320200]

32 **Tirosh A**, Garg R, Adler GK. Mineralocorticoid receptor antagonists and the metabolic syndrome. *Curr Hypertens Rep* 2010; **12**: 252-257 [PMID: 20563672 DOI: 10.1007/s11906-010-0126-2]

33 **Nigro J**, Osman N, Dart AM, Little PJ. Insulin resistance and atherosclerosis. *Endocr Rev* 2006; **27**: 242-259 [PMID: 16492903 DOI: 10.1210/er.2005-0007]

34 **Sell H**, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. *Nat Rev Endocrinol* 2012; **8**: 709-716 [PMID: 22847239 DOI: 10.1038/nrendo.2012.114]

35 **Nishida K**, Otsu K. Inflammation and metabolic cardiomyopathy. *Cardiovasc Res* 2017; **113**: 389-398 [PMID: 28395010 DOI: 10.1093/cvr/cvx012]

36 **Yu Q**, Vazquez R, Zabadi S, Watson RR, Larson DF. T-lymphocytes mediate left ventricular fibrillar collagen cross-linking and diastolic dysfunction in mice. *Matrix Biol* 2010; **29**: 511-518 [PMID: 20600894 DOI: 10.1016/j.matbio.2010.06.003]

37 **Cao Y**, Xu W, Xiong S. Adoptive transfer of regulatory T cells protects against Coxsackievirus B3-induced cardiac fibrosis. *PLoS One* 2013; **8**: e74955 [PMID: 24023968 DOI: 10.1371/journal.pone.0074955]

38 **Schaffer SW**. Cardiomyopathy associated with noninsulin-dependent diabetes. *Mol Cell Biochem* 1991; **107**: 1-20 [PMID: 1664489 DOI: 10.1007/BF02424571]

39 **Nagoshi T**, Yoshimura M, Rosano GM, Lopaschuk GD, Mochizuki S. Optimization of cardiac metabolism in heart failure. *Curr Pharm Des* 2011; **17**: 3846-3853 [PMID: 21933140 DOI: 10.2174/138161211798357773]

40 **Tuunanen H**, Knuuti J. Metabolic remodelling in human heart failure. *Cardiovasc Res* 2011; **90**: 251-257 [PMID: 21372005 DOI: 10.1093/cvr/cvr052]

41 **Bando YK**, Murohara T. Diabetes-related heart failure. *Circ J* 2014; **78**: 576-583 [PMID: 24500073 DOI: 10.1253/circj.cj-13-1564]

42 **Thomas MC**, Iyngkaran P. Forensic interrogation of diabetic endothelitis in cardiovascular diseases and clinical translation in heart failure. *World J Cardiol* 2020; **12**: 409-418 [PMID: 32879703 DOI: 10.4330/wjc.v12.i8.409]

43 **Joubert M**, Jagu B, Montaigne D, Marechal X, Tesse A, Ayer A, Dollet L, Le May C, Toumaniantz G, Manrique A, Charpentier F, Staels B, Magré J, Cariou B, Prieur X. The Sodium-Glucose Cotransporter 2 Inhibitor Dapagliflozin Prevents Cardiomyopathy in a Diabetic Lipodystrophic Mouse Model. *Diabetes* 2017; **66**: 1030-1040 [PMID: 28052965 DOI: 10.2337/db16-0733]

44 **Arow M**, Waldman M, Yadin D, Nudelman V, Shainberg A, Abraham NG, Freimark D, Kornowski R, Aravot D, Hochhauser E, Arad M. Sodium-glucose cotransporter 2 inhibitor Dapagliflozin attenuates diabetic cardiomyopathy. *Cardiovasc Diabetol* 2020; **19**: 7 [PMID: 31924211 DOI: 10.1186/s12933-019-0980-4]

45 **Alkagiet S**, Tziomalos K. Role of sodium-glucose co-transporter-2 inhibitors in the management of heart failure in patients with diabetes mellitus. *World J Diabetes* 2020; **11**: 150-154 [PMID: 32477451 DOI: 10.4239/wjd.v11.i5.150]

46 **Zhao J**, Randive R, Stewart JA. Molecular mechanisms of AGE/RAGE-mediated fibrosis in the diabetic heart. *World J Diabetes* 2014; **5**: 860-867 [PMID: 25512788 DOI: 10.4239/wjd.v5.i6.860]

47 **Wendt T**, Bucciarelli L, Qu W, Lu Y, Yan SF, Stern DM, Schmidt AM. Receptor for advanced glycation endproducts (RAGE) and vascular inflammation: insights into the pathogenesis of macrovascular complications in diabetes. *Curr Atheroscler Rep* 2002; **4**: 228-237 [PMID: 11931721 DOI: 10.1007/s11883-002-0024-4]

48 **Dhalla NS**, Liu X, Panagia V, Takeda N. Subcellular remodeling and heart dysfunction in chronic diabetes. *Cardiovasc Res* 1998; **40**: 239-247 [PMID: 9893715 DOI: 10.1016/s0008-6363(98)00186-2]

49 **Garcia NA**, Moncayo-Arlandi J, Sepulveda P, Diez-Juan A. Cardiomyocyte exosomes regulate glycolytic flux in endothelium by direct transfer of GLUT transporters and glycolytic enzymes. *Cardiovasc Res* 2016; **109**: 397-408 [PMID: 26609058 DOI: 10.1093/cvr/cvv260]

50 **Falcão-Pires I**, Leite-Moreira AF. Diabetic cardiomyopathy: understanding the molecular and cellular basis to progress in diagnosis and treatment. *Heart Fail Rev* 2012; **17**: 325-344 [PMID: 21626163 DOI: 10.1007/s10741-011-9257-z]

51 **Bostick B**, Habibi J, Ma L, Aroor A, Rehmer N, Hayden MR, Sowers JR. Dipeptidyl peptidase

inhibition prevents diastolic dysfunction and reduces myocardial fibrosis in a mouse model of Western diet induced obesity. *Metabolism* 2014; **63**: 1000-1011 [PMID: 24933400 DOI: 10.1016/j.metabol.2014.04.002]

52 **Bostick B**, Habibi J, DeMarco VG, Jia G, Domeier TL, Lambert MD, Aroor AR, Nistala R, Bender SB, Garro M, Hayden MR, Ma L, Manrique C, Sowers JR. Mineralocorticoid receptor blockade prevents Western diet-induced diastolic dysfunction in female mice. *Am J Physiol Heart Circ Physiol* 2015; **308**: H1126-H1135 [PMID: 25747754 DOI: 10.1152/ajpheart.00898.2014]

53 **Westermeier F**, Riquelme JA, Pavez M, Garrido V, Díaz A, Verdejo HE, Castro PF, García L, Lavandero S. New Molecular Insights of Insulin in Diabetic Cardiomyopathy. *Front Physiol* 2016; **7**: 125 [PMID: 27148064 DOI: 10.3389/fphys.2016.00125]

54 **Ernande L**, Derumeaux G. Diabetic cardiomyopathy: myth or reality? *Arch Cardiovasc Dis* 2012; **105**: 218-225 [PMID: 22633296 DOI: 10.1016/j.acvd.2011.11.007]

55 **Harper AR**, Patel HC, Lyon AR. Heart failure with preserved ejection fraction. *Clin Med (Lond)* 2018; **18**: s24-s29 [PMID: 29700089 DOI: 10.7861/clinmedicine.18-2-s24]

56 **Borghetti G**, von Lewinski D, Eaton DM, Sourij H, Houser SR, Wallner M. Diabetic Cardiomyopathy: Current and Future Therapies. Beyond Glycemic Control. *Front Physiol* 2018; **9**: 1514 [PMID: 30425649 DOI: 10.3389/fphys.2018.01514]

57 **Inoue Y**, Kawai M, Minai K, Ogawa K, Nagoshi T, Ogawa T, Yoshimura M. The impact of an inverse correlation between plasma B-type natriuretic peptide levels and insulin resistance on the diabetic condition in patients with heart failure. *Metabolism* 2016; **65**: 38-47 [PMID: 26892514 DOI: 10.1016/j.metabol.2015.09.019]

58 **Howarth FC**, Al-Shamsi N, Al-Qaydi M, Al-Mazrouei M, Qureshi A, Chandranath SI, Kazzam E, Adem A. Effects of brain natriuretic peptide on contraction and intracellular Ca2+ in ventricular myocytes from the streptozotocin-induced diabetic rat. *Ann N Y Acad Sci* 2006; **1084**: 155-165 [PMID: 17151299 DOI: 10.1196/annals.1372.007]

59 **Howarth FC**, Adem A, Adeghate EA, Al Ali NA, Al Bastaki AM, Sorour FR, Hammoudi RO, Ghaleb NA, Chandler NJ, Dobrzynski H. Distribution of atrial natriuretic peptide and its effects on contraction and intracellular calcium in ventricular myocytes from streptozotocin-induced diabetic rat. *Peptides* 2005; **26**: 691-700 [PMID: 15752585 DOI: 10.1016/j.peptides.2004.12.003]

60 **Dahlström U**. Can natriuretic peptides be used for the diagnosis of diastolic heart failure? *Eur J Heart Fail* 2004; **6**: 281-287 [PMID: 14987577 DOI: 10.1016/j.ejheart.2004.01.005]

61 **Betti I**, Castelli G, Barchielli A, Beligni C, Boscherini V, De Luca L, Messeri G, Gheorghiade M, Maisel A, Zuppiroli A. The role of N-terminal PRO-brain natriuretic peptide and echocardiography for screening asymptomatic left ventricular dysfunction in a population at high risk for heart failure. The PROBE-HF study. *J Card Fail* 2009; **15**: 377-384 [PMID: 19477397 DOI: 10.1016/j.cardfail.2008.12.002]

62 **Dencker M**, Stagmo M, Dorkhan M. Relationship between natriuretic peptides and echocardiography parameters in patients with poorly regulated type 2 diabetes. *Vasc Health Risk Manag* 2010; **6**: 373-382 [PMID: 20539839 DOI: 10.2147/vhrm.s9332]

63 **Valle R**, Bagolin E, Canali C, Giovinazzo P, Barro S, Aspromonte N, Carbonieri E, Milani L. The BNP assay does not identify mild left ventricular diastolic dysfunction in asymptomatic diabetic patients. *Eur J Echocardiogr* 2006; **7**: 40-44 [PMID: 15886060 DOI: 10.1016/j.euje.2005.03.009]

64 **Brunner-La Rocca HP**, Sanders-van Wijk S. Natriuretic Peptides in Chronic Heart Failure. *Card Fail Rev* 2019; **5**: 44-49 [PMID: 30847245 DOI: 10.15420/cfr.2018.26.1]

65 **Romano S**, Di Mauro M, Fratini S, Guarracini L, Guarracini F, Poccia G, Penco M. Early diagnosis of left ventricular diastolic dysfunction in diabetic patients: a possible role for natriuretic peptides. *Cardiovasc Diabetol* 2010; **9**: 89 [PMID: 21162718 DOI: 10.1186/1475-2840-9-89]

66 **Rahimi K**, Bennett D, Conrad N, Williams TM, Basu J, Dwight J, Woodward M, Patel A, McMurray J, MacMahon S. Risk prediction in patients with heart failure: a systematic review and analysis. *JACC Heart Fail* 2014; **2**: 440-446 [PMID: 25194291 DOI: 10.1016/j.jchf.2014.04.008]

67 **Gomes AV**, Potter JD, Szczesna-Cordary D. The role of troponins in muscle contraction. *IUBMB Life* 2002; **54**: 323-333 [PMID: 12665242 DOI: 10.1080/15216540216037]

68 **Russell NE**, Higgins MF, Amaruso M, Foley M, McAuliffe FM. Troponin T and pro-B-type natriuretic Peptide in fetuses of type 1 diabetic mothers. *Diabetes Care* 2009; **32**: 2050-2055 [PMID: 19690080 DOI: 10.2337/dc09-0552]

69 **Malhotra A**, Kang BP, Cheung S, Opawumi D, Meggs LG. Angiotensin II promotes glucose-induced activation of cardiac protein kinase C isozymes and phosphorylation of troponin I. *Diabetes* 2001; **50**: 1918-1926 [PMID: 11473056 DOI: 10.2337/diabetes.50.8.1918]

70 **Hange-Dickerson PA**. Oncology nurse practitioner provides continuity of care. *Nurse Pract* 1992; **17**: 14 [PMID: 1608567 DOI: 10.1152/ajpheart.00638.2005]

71 **Malhotra A**, Reich D, Nakouzi A, Sanghi V, Geenen DL, Buttrick PM. Experimental diabetes is associated with functional activation of protein kinase C epsilon and phosphorylation of troponin I in the heart, which are prevented by angiotensin II receptor blockade. *Circ Res* 1997; **81**: 1027-1033 [PMID: 9400384 DOI: 10.1161/01.res.81.6.1027]

72 **Eggers KM**, Al-Shakarchi J, Berglund L, Lindahl B, Siegbahn A, Wallentin L, Zethelius B. High-sensitive cardiac troponin T and its relations to cardiovascular risk factors, morbidity, and mortality in elderly men. *Am Heart J* 2013; **166**: 541-548 [PMID: 24016505 DOI: 10.1016/j.ahj.2013.07.004]

73 **Segre CA**, Hueb W, Garcia RM, Rezende PC, Favarato D, Strunz CM, Sprandel Mda C, Roggério A, Carvalho AL, Maranhão RC, Ramires JA, Kalil Filho R. Troponin in diabetic patients with and

without chronic coronary artery disease. *BMC Cardiovasc Disord* 2015; **15**: 72 [PMID: 26195004 DOI: 10.1186/s12872-015-0051-z]

74 **Lopez-Andres N**, Fortuno MA, Diez J, Zannad F, Lacolley P, Rossignol P. Vascular effects of cardiotrophin-1: a role in hypertension? *J Hypertens* 2010; **28**: 1261-1272 [PMID: 20216087 DOI: 10.1097/HJH.0b013e328337fe42]

75 **Hishinuma S**, Funamoto M, Fujio Y, Kunisada K, Yamauchi-Takihara K. Hypoxic stress induces cardiotrophin-1 expression in cardiac myocytes. *Biochem Biophys Res Commun* 1999; **264**: 436-440 [PMID: 10529382 DOI: 10.1006/bbrc.1999.1535]

76 **Robador PA**, San José G, Rodríguez C, Guadall A, Moreno MU, Beaumont J, Fortuño A, Díez J, Martínez-González J, Zalba G. HIF-1-mediated up-regulation of cardiotrophin-1 is involved in the survival response of cardiomyocytes to hypoxia. *Cardiovasc Res* 2011; **92**: 247-255 [PMID: 21771897 DOI: 10.1093/cvr/cvr202]

77 **Ateghang B**, Wartenberg M, Gassmann M, Sauer H. Regulation of cardiotrophin-1 expression in mouse embryonic stem cells by HIF-1alpha and intracellular reactive oxygen species. *J Cell Sci* 2006; **119**: 1043-1052 [PMID: 16507596 DOI: 10.1242/jcs.02798]

78 **Fukuzawa J**, Booz GW, Hunt RA, Shimizu N, Karoor V, Baker KM, Dostal DE. Cardiotrophin-1 increases angiotensinogen mRNA in rat cardiac myocytes through STAT3: an autocrine loop for hypertrophy. *Hypertension* 2000; **35**: 1191-1196 [PMID: 10856262 DOI: 10.1161/01.hyp.35.6.1191]

79 **López-Andrés N**, Iñigo C, Gallego I, Díez J, Fortuño MA. Aldosterone induces cardiotrophin-1 expression in HL-1 adult cardiomyocytes. *Endocrinology* 2008; **149**: 4970-4978 [PMID: 18566129 DOI: 10.1210/en.2008-0120]

80 **Janjua S**, Lawrence KM, Ng LL, Latchman DS. The cardioprotective agent urocortin induces expression of CT-1. *Cardiovasc Toxicol* 2003; **3**: 255-262 [PMID: 14555790 DOI: 10.1385/ct:3:3:255]

81 **Liu J**, Liu Z, Huang F, Xing Z, Wang H, Li Z. Pioglitazone inhibits hypertrophy induced by high glucose and insulin in cultured neonatal rat cardiomyocytes. *Pharmazie* 2007; **62**: 925-929 [PMID: 18214344]

82 **Jiang ZS**, Jeyaraman M, Wen GB, Fandrich RR, Dixon IM, Cattini PA, Kardami E. High- but not low-molecular weight FGF-2 causes cardiac hypertrophy in vivo; possible involvement of cardiotrophin-1. *J Mol Cell Cardiol* 2007; **42**: 222-233 [PMID: 17045289 DOI: 10.1016/j.yjmcc.2006.09.002]

83 **Ruiz-Hurtado G**, Gómez-Hurtado N, Fernández-Velasco M, Calderón E, Smani T, Ordoñez A, Cachofeiro V, Boscá L, Díez J, Gómez AM, Delgado C. Cardiotrophin-1 induces sarcoplasmic reticulum Ca(2+) leak and arrhythmogenesis in adult rat ventricular myocytes. *Cardiovasc Res* 2012; **96**: 81-89 [PMID: 22787135 DOI: 10.1093/cvr/cvs234]

84 **Jougasaki M**. Cardiotrophin-1 in cardiovascular regulation. *Adv Clin Chem* 2010; **52**: 41-76 [PMID: 21275339 DOI: 10.1016/s0065-2423(10)52002-x]

85 **García-Cenador MB**, Lopez-Novoa JM, Díez J, García-Criado FJ. Effects and mechanism of organ protection by cardiotrophin-1. *Curr Med Chem* 2013; **20**: 246-256 [PMID: 23244580 DOI: 10.2174/092986713804806702]

86 **Moreno-Aliaga MJ**, Romero-Lozano MA, Castaño D, Prieto J, Bustos M. Role of cardiotrophin-1 in obesity and insulin resistance. *Adipocyte* 2012; **1**: 112-115 [PMID: 23700521 DOI: 10.4161/adip.19696]

87 **Gamella-Pozuelo L**, Fuentes-Calvo I, Gómez-Marcos MA, Recio-Rodriguez JI, Agudo-Conde C, Fernández-Martín JL, Cannata-Andía JB, López-Novoa JM, García-Ortiz L, Martínez-Salgado C. Plasma Cardiotrophin-1 as a Marker of Hypertension and Diabetes-Induced Target Organ Damage and Cardiovascular Risk. *Medicine (Baltimore)* 2015; **94**: e1218 [PMID: 26222851 DOI: 10.1097/MD.0000000000001218]

88 **Briana DD**, Germanou K, Boutsikou M, Boutsikou T, Athanasopoulos N, Marmarinos A, Gourgiotis D, Malamitsi-Puchner A. Potential prognostic biomarkers of cardiovascular disease in fetal macrosomia: the impact of gestational diabetes. *J Matern Fetal Neonatal Med* 2018; **31**: 895-900 [PMID: 28298172 DOI: 10.1080/14767058.2017.1300651]

89 **van den Brom CE**, Huisman MC, Vlasblom R, Boontje NM, Duijst S, Lubberink M, Molthoff CF, Lammertsma AA, van der Velden J, Boer C, Ouwens DM, Diamant M. Altered myocardial substrate metabolism is associated with myocardial dysfunction in early diabetic cardiomyopathy in rats: studies using positron emission tomography. *Cardiovasc Diabetol* 2009; **8**: 39 [PMID: 19624828 DOI: 10.1186/1475-2840-8-39]

90 **Hung HC**, Lu FH, Ou HY, Wu HT, Wu JS, Yang YC, Chang CJ. Increased cardiotrophin-1 in subjects with impaired glucose tolerance and newly diagnosed diabetes. *Int J Cardiol* 2013; **169**: e33-e34 [PMID: 24054317 DOI: 10.1016/j.ijcard.2013.08.112]

91 **Pennica D**, King KL, Shaw KJ, Luis E, Rullamas J, Luoh SM, Darbonne WC, Knutzon DS, Yen R, Chien KR. Expression cloning of cardiotrophin 1, a cytokine that induces cardiac myocyte hypertrophy. *Proc Natl Acad Sci USA* 1995; **92**: 1142-1146 [PMID: 7862649 DOI: 10.1073/pnas.92.4.1142]

92 **Rendo-Urteaga T**, García-Calzón S, Martínez-Ansó E, Chueca M, Oyarzabal M, Azcona-Sanjulián MC, Bustos M, Moreno-Aliaga MJ, Martínez JA, Marti A. Decreased cardiotrophin-1 Levels are associated with a lower risk of developing the metabolic syndrome in overweight/obese children after a weight loss program. *Metabolism* 2013; **62**: 1429-1436 [PMID: 23856329 DOI: 10.1016/j.metabol.2013.05.011]

93 **Gritman K**, Van Winkle DM, Lorentz CU, Pennica D, Habecker BA. The lack of cardiotrophin-1 alters expression of interleukin-6 and leukemia inhibitory factor mRNA but does not impair cardiac injury response. *Cytokine* 2006; **36**: 9-16 [PMID: 17150369 DOI: 10.1016/j.cyto.2006.10.004]

94 **Hwa V**, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr Rev* 1999; **20**: 761-787 [PMID: 10605625 DOI: 10.1210/edrv.20.6.0382]

95 **López-Bermejo A**, Khosravi J, Fernández-Real JM, Hwa V, Pratt KL, Casamitjana R, Garcia-Gil MM, Rosenfeld RG, Ricart W. Insulin resistance is associated with increased serum concentration of IGF-binding protein-related protein 1 (IGFBP-rP1/MAC25). *Diabetes* 2006; **55**: 2333-2339 [PMID: 16873698 DOI: 10.2337/db05-1627]

96 **Kutsukake M**, Ishihara R, Momose K, Isaka K, Itokazu O, Higuma C, Matsutani T, Matsuda A, Sasajima K, Hara T, Tamura K. Circulating IGF-binding protein 7 (IGFBP7) levels are elevated in patients with endometriosis or undergoing diabetic hemodialysis. *Reprod Biol Endocrinol* 2008; **6**: 54 [PMID: 19019211 DOI: 10.1186/1477-7827-6-54]

97 **Liu Y**, Wu M, Ling J, Cai L, Zhang D, Gu HF, Wang H, Zhu Y, Lai M. Serum IGFBP7 Levels associate with insulin resistance and the risk of metabolic syndrome in a Chinese population. *Sci Rep* 2015; **5**: 10227 [PMID: 25984973 DOI: 10.1038/srep10227]

98 **Vizioli MG**, Sensi M, Miranda C, Cleris L, Formelli F, Anania MC, Pierotti MA, Greco A. IGFBP7: an oncosuppressor gene in thyroid carcinogenesis. *Oncogene* 2010; **29**: 3835-3844 [PMID: 20440262 DOI: 10.1038/onc.2010.136]

99 **Guo XH**, Liu LX, Zhang HY, Zhang QQ, Li Y, Tian XX, Qiu ZH. Insulin-like growth factor binding protein-related protein 1 contributes to hepatic fibrogenesis. *J Dig Dis* 2014; **15**: 202-210 [PMID: 24373620 DOI: 10.1111/1751-2980.12126]

100 **Gandhi PU**, Gaggin HK, Sheftel AD, Belcher AM, Weiner RB, Baggish AL, Motiwala SR, Liu PP, Januzzi JL Jr. Prognostic usefulness of insulin-like growth factor-binding protein 7 in heart failure with reduced ejection fraction: a novel biomarker of myocardial diastolic function? *Am J Cardiol* 2014; **114**: 1543-1549 [PMID: 25248814 DOI: 10.1016/j.amjcard.2014.08.018]

101 **Kalayci A**, Peacock WF, Nagurney JT, Hollander JE, Levy PD, Singer AJ, Shapiro NI, Cheng RK, Cannon CM, Blomkalns AL, Walters EL, Christenson RH, Chen-Tournoux A, Nowak RM, Lurie MD, Pang PS, Kastner P, Masson S, Gibson CM, Gaggin HK, Januzzi JL Jr. Echocardiographic assessment of insulin-like growth factor binding protein-7 and early identification of acute heart failure. *ESC Heart Fail* 2020; **7**: 1664-1675 [PMID: 32406612 DOI: 10.1002/ehf2.12722]

102 **Shaver A**, Nichols A, Thompson E, Mallick A, Payne K, Jones C, Manne ND, Sundaram S, Shapiro JI, Sodhi K. Role of Serum Biomarkers in Early Detection of Diabetic Cardiomyopathy in the West Virginian Population. *Int J Med Sci* 2016; **13**: 161-168 [PMID: 26941576 DOI: 10.7150/ijms.14141]

103 **Ziyadeh FN**, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, Chen S, McGowan TA, Sharma K. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. *Proc Natl Acad Sci USA* 2000; **97**: 8015-8020 [PMID: 10859350 DOI: 10.1073/pnas.120055097]

104 **Iglesias-De La Cruz MC**, Ruiz-Torres P, Alcamí J, Díez-Marqués L, Ortega-Velázquez R, Chen S, Rodríguez-Puyol M, Ziyadeh FN, Rodríguez-Puyol D. Hydrogen peroxide increases extracellular matrix mRNA through TGF-beta in human mesangial cells. *Kidney Int* 2001; **59**: 87-95 [PMID: 11135061 DOI: 10.1046/j.1523-1755.2001.00469.x]

105 **Asbun J**, Villarreal FJ. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. *J Am Coll Cardiol* 2006; **47**: 693-700 [PMID: 16487830 DOI: 10.1016/j.jacc.2005.09.050]

106 **Pohlers D**, Brenmoehl J, Löffler I, Müller CK, Leipner C, Schultze-Mosgau S, Stallmach A, Kinne RW, Wolf G. TGF-beta and fibrosis in different organs - molecular pathway imprints. *Biochim Biophys Acta* 2009; **1792**: 746-756 [PMID: 19539753 DOI: 10.1016/j.bbadis.2009.06.004]

107 **Wermuth PJ**, Carney KR, Mendoza FA, Piera-Velazquez S, Jimenez SA. Endothelial cell-specific activation of transforming growth factor-β signaling in mice induces cutaneous, visceral, and microvascular fibrosis. *Lab Invest* 2017; **97**: 806-818 [PMID: 28346399 DOI: 10.1038/Labinvest.2017.23]

108 **Pardali E**, Sanchez-Duffhues G, Gomez-Puerto MC, Ten Dijke P. TGF-β-Induced Endothelial-Mesenchymal Transition in Fibrotic Diseases. *Int J Mol Sci* 2017; **18** [PMID: 29039786 DOI: 10.3390/ijms18102157]

109 **Tan SM**, Zhang Y, Wang B, Tan CY, Zammit SC, Williams SJ, Krum H, Kelly DJ. FT23, an orally active antifibrotic compound, attenuates structural and functional abnormalities in an experimental model of diabetic cardiomyopathy. *Clin Exp Pharmacol Physiol* 2012; **39**: 650-656 [PMID: 22612418 DOI: 10.1111/j.1440-1681.2012.05726.x]

110 **Bujak M**, Ren G, Kweon HJ, Dobaczewski M, Reddy A, Taffet G, Wang XF, Frangogiannis NG. Essential role of Smad3 in infarct healing and in the pathogenesis of cardiac remodeling. *Circulation* 2007; **116**: 2127-2138 [PMID: 17967775 DOI: 10.1161/CIRCULATIONAHA.107.704197]

111 **Biernacka A**, Cavalera M, Wang J, Russo I, Shinde A, Kong P, Gonzalez-Quesada C, Rai V, Dobaczewski M, Lee DW, Wang XF, Frangogiannis NG. Smad3 Signaling Promotes Fibrosis While Preserving Cardiac and Aortic Geometry in Obese Diabetic Mice. *Circ Heart Fail* 2015; **8**: 788-798 [PMID: 25985794 DOI: 10.1161/CIRCHEARTFAILURE.114.001963]

112 **Greulich S**, Maxhera B, Vandenplas G, de Wiza DH, Smiris K, Mueller H, Heinrichs J, Blumensatt M, Cuvelier C, Akhyari P, Ruige JB, Ouwens DM, Eckel J. Secretory products from epicardial

adipose tissue of patients with type 2 diabetes mellitus induce cardiomyocyte dysfunction. *Circulation* 2012; **126**: 2324-2334 [PMID: 23065384 DOI: 10.1161/CIRCULATIONAHA.111.039586]

113 **Greulich S**, de Wiza DH, Preilowski S, Ding Z, Mueller H, Langin D, Jaquet K, Ouwens DM, Eckel J. Secretory products of guinea pig epicardial fat induce insulin resistance and impair primary adult rat cardiomyocyte function. *J Cell Mol Med* 2011; **15**: 2399-2410 [PMID: 21143387 DOI: 10.1111/j.1582-4934.2010.01232.x]

114 **Ouwens DM**, Diamant M. Myocardial insulin action and the contribution of insulin resistance to the pathogenesis of diabetic cardiomyopathy. *Arch Physiol Biochem* 2007; **113**: 76-86 [PMID: 17558606 DOI: 10.1080/13813450701422633]

115 **Blumensatt M**, Greulich S, Herzfeld de Wiza D, Mueller H, Maxhera B, Rabelink MJ, Hoeben RC, Akhyari P, Al-Hasani H, Ruige JB, Ouwens DM. Activin A impairs insulin action in cardiomyocytes *via* up-regulation of miR-143. *Cardiovasc Res* 2013; **100**: 201-210 [PMID: 23812417 DOI: 10.1093/cvr/cvt173]

116 **Chen WJ**, Greulich S, van der Meer RW, Rijzewijk LJ, Lamb HJ, de Roos A, Smit JW, Romijn JA, Ruige JB, Lammertsma AA, Lubberink M, Diamant M, Ouwens DM. Activin A is associated with impaired myocardial glucose metabolism and left ventricular remodeling in patients with uncomplicated type 2 diabetes. *Cardiovasc Diabetol* 2013; **12**: 150 [PMID: 24134550 DOI: 10.1186/1475-2840-12-150]

117 **Yndestad A**, Ueland T, Øie E, Florholmen G, Halvorsen B, Attramadal H, Simonsen S, Frøland SS, Gullestad L, Christensen G, Damås JK, Aukrust P. Elevated levels of activin A in heart failure: potential role in myocardial remodeling. *Circulation* 2004; **109**: 1379-1385 [PMID: 14993131 DOI: 10.1161/01.CIR.0000120704.97934.41]

118 **Ueland T**, Aukrust P, Aakhus S, Smith C, Endresen K, Birkeland KI, Gullestad L, Johansen OE. Activin A and cardiovascular disease in type 2 diabetes mellitus. *Diab Vasc Dis Res* 2012; **9**: 234-237 [PMID: 22234949 DOI: 10.1177/1479164111431171]

119 **Weigert J**, Neumeier M, Wanninger J, Schober F, Sporrer D, Weber M, Schramm A, Wurm S, Stögbauer F, Filarsky M, Schäffler A, Aslanidis C, Schölmerich J, Buechler C. Adiponectin upregulates monocytic activin A but systemic levels are not altered in obesity or type 2 diabetes. *Cytokine* 2009; **45**: 86-91 [PMID: 19128983 DOI: 10.1016/j.cyto.2008.10.017]

120 **Wu H**, Wu M, Chen Y, Allan CA, Phillips DJ, Hedger MP. Correlation between blood activin levels and clinical parameters of type 2 diabetes. *Exp Diabetes Res* 2012; **2012**: 410579 [PMID: 23304117 DOI: 10.1155/2012/410579]

121 **Venteclef N**, Guglielmi V, Balse E, Gaborit B, Cotillard A, Atassi F, Amour J, Leprince P, Dutour A, Clément K, Hatem SN. Human epicardial adipose tissue induces fibrosis of the atrial myocardium through the secretion of adipo-fibrokines. *Eur Heart J* 2015; **36**: 795-805a [PMID: 23525094 DOI: 10.1093/eurheartj/eht099]

122 **Li N**, Wu H, Geng R, Tang Q. Identification of Core Gene Biomarkers in Patients with Diabetic Cardiomyopathy. *Dis Markers* 2018; **2018**: 6025061 [PMID: 30662576 DOI: 10.1155/2018/6025061]

123 **Berezin AE**. Diabetes mellitus related biomarker: The predictive role of growth-differentiation factor-15. *Diabetes Metab Syndr* 2016; **10**: S154-S157 [PMID: 26482961 DOI: 10.1016/j.dsx.2015.09.016]

124 **Dostálová I**, Roubícek T, Bártlová M, Mráz M, Lacinová Z, Haluzíková D, Kaválková P, Matoulek M, Kasalicky M, Haluzík M. Increased serum concentrations of macrophage inhibitory cytokine-1 in patients with obesity and type 2 diabetes mellitus: the influence of very low calorie diet. *Eur J Endocrinol* 2009; **161**: 397-404 [PMID: 19515791 DOI: 10.1530/EJE-09-0417]

125 **Daniels LB**, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo Study. *Circulation* 2011; **123**: 2101-2110 [PMID: 21536998 DOI: 10.1161/CIRCULATIONAHA.110.979740]

126 **Lind L**, Wallentin L, Kempf T, Tapken H, Quint A, Lindahl B, Olofsson S, Venge P, Larsson A, Hulthe J, Elmgren A, Wollert KC. Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study. *Eur Heart J* 2009; **30**: 2346-2353 [PMID: 19561023 DOI: 10.1093/eurheartj/ehp261]

127 **Schnabel RB**, Schulz A, Messow CM, Lubos E, Wild PS, Zeller T, Sinning CR, Rupprecht HJ, Bickel C, Peetz D, Cambien F, Kempf T, Wollert KC, Benjamin EJ, Lackner KJ, Münzel TF, Tiret L, Vasan RS, Blankenberg S. Multiple marker approach to risk stratification in patients with stable coronary artery disease. *Eur Heart J* 2010; **31**: 3024-3031 [PMID: 20852293 DOI: 10.1093/eurheartj/ehq322]

128 **Baessler A**, Strack C, Rousseva E, Wagner F, Bruxmeier J, Schmiedel M, Riegger G, Lahmann C, Loew T, Schmitz G, Fischer M. Growth-differentiation factor-15 improves reclassification for the diagnosis of heart failure with normal ejection fraction in morbid obesity. *Eur J Heart Fail* 2012; **14**: 1240-1248 [PMID: 22843563 DOI: 10.1093/eurjhf/hfs116]

129 **Stahrenberg R**, Edelmann F, Mende M, Kockskämper A, Düngen HD, Lüers C, Binder L, Herrmann-Lingen C, Gelbrich G, Hasenfuss G, Pieske B, Wachter R. The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. *Eur J Heart Fail* 2010; **12**: 1309-1316 [PMID: 20837635 DOI: 10.1093/eurjhf/hfq151]

130 **Dominguez-Rodriguez A**, Abreu-Gonzalez P, Avanzas P. Usefulness of growth differentiation factor-15 Levels to predict diabetic cardiomyopathy in asymptomatic patients with type 2 diabetes mellitus. *Am J Cardiol* 2014; **114**: 890-894 [PMID: 25073564 DOI: 10.1016/j.amjcard.2014.06.020]

131 **Lu J**, Zhang Y, Dong X, Lu J, Zhang C, Liu J, Yu Q, Teng H, Yao Q, Yin J, Qin L. Association between MIC-1 and Type 2 Diabetes: A Combined Analysis. *Dis Markers* 2019; **2019**: 7284691 [PMID: 31827641 DOI: 10.1155/2019/7284691]

132 **Tang M**, Luo M, Lu W, Wang S, Zhang R, Liang W, Gu J, Yu X, Zhang X, Hu C. Serum growth differentiation factor 15 is associated with glucose metabolism in the third trimester in Chinese pregnant women. *Diabetes Res Clin Pract* 2019; **156**: 107823 [PMID: 31446114 DOI: 10.1016/j.diabres.2019.107823]

133 **Tsai VWW**, Husaini Y, Sainsbury A, Brown DA, Breit SN. The MIC-1/GDF15-GFRAL Pathway in Energy Homeostasis: Implications for Obesity, Cachexia, and Other Associated Diseases. *Cell Metab* 2018; **28**: 353-368 [PMID: 30184485 DOI: 10.1016/j.cmet.2018.07.018]

134 **Suthahar N**, Meijers WC, Silljé HHW, Ho JE, Liu FT, de Boer RA. Galectin-3 Activation and Inhibition in Heart Failure and Cardiovascular Disease: An Update. *Theranostics* 2018; **8**: 593-609 [PMID: 29344292 DOI: 10.7150/thno.22196]

135 **Tan KCB**, Cheung CL, Lee ACH, Lam JKY, Wong Y, Shiu SWM. Galectin-3 is independently associated with progression of nephropathy in type 2 diabetes mellitus. *Diabetologia* 2018; **61**: 1212-1219 [PMID: 29417184 DOI: 10.1007/s00125-018-4552-z]

136 **Luís C**, Costa R, Rodrigues I, Castela Â, Coelho P, Guerreiro S, Gomes J, Reis C, Soares R. Xanthohumol and 8-prenylnaringenin reduce type 2 diabetes-associated oxidative stress by downregulating galectin-3. *Porto Biomed J* 2019; **4**: e23 [PMID: 31595252 DOI: 10.1016/j.pbj.0000000000000023]

137 **Berezin A**. Metabolic memory phenomenon in diabetes mellitus: Achieving and perspectives. *Diabetes Metab Syndr* 2016; **10**: S176-S183 [PMID: 27025794 DOI: 10.1016/j.dsx.2016.03.016]

138 **Song X**, Qian X, Shen M, Jiang R, Wagner MB, Ding G, Chen G, Shen B. Protein kinase C promotes cardiac fibrosis and heart failure by modulating galectin-3 expression. *Biochim Biophys Acta* 2015; **1853**: 513-521 [PMID: 25489662 DOI: 10.1016/j.bbamcr.2014.12.001]

139 **Azibani F**, Benard L, Schlossarek S, Merval R, Tournoux F, Fazal L, Polidano E, Launay JM, Carrier L, Chatziantoniou C, Samuel JL, Delcayre C. Aldosterone inhibits antifibrotic factors in mouse hypertensive heart. *Hypertension* 2012; **59**: 1179-1187 [PMID: 22547442 DOI: 10.1161/HYPERTENSIONAHA.111.190512]

140 **Calvier L**, Miana M, Reboul P, Cachofeiro V, Martinez-Martinez E, de Boer RA, Poirier F, Lacolley P, Zannad F, Rossignol P, López-Andrés N. Galectin-3 mediates aldosterone-induced vascular fibrosis. *Arterioscler Thromb Vasc Biol* 2013; **33**: 67-75 [PMID: 23117656 DOI: 10.1161/ATVBAHA.112.300569]

141 **Ibarrola J**, Sádaba R, Garcia-Peña A, Arrieta V, Martinez-Martinez E, Alvarez V, Fernández-Celis A, Gainza A, Santamaría E, Fernández-Irigoyen J, Cachofeiro V, Fay R, Rossignol P, López-Andrés N. A role for fumarate hydratase in mediating oxidative effects of galectin-3 in human cardiac fibroblasts. *Int J Cardiol* 2018; **258**: 217-223 [PMID: 29544935 DOI: 10.1016/j.ijcard.2017.12.103]

142 **Ibarrola J**, Arrieta V, Sádaba R, Martinez-Martinez E, Garcia-Peña A, Alvarez V, Fernández-Celis A, Gainza A, Santamaría E, Fernández-Irigoyen J, Cachofeiro V, Zalba G, Fay R, Rossignol P, López-Andrés N. Galectin-3 down-regulates antioxidant peroxiredoxin-4 in human cardiac fibroblasts: a new pathway to induce cardiac damage. *Clin Sci (Lond)* 2018; **132**: 1471-1485 [PMID: 29674526 DOI: 10.1042/CS20171389]

143 **He J**, Li X, Luo H, Li T, Zhao L, Qi Q, Liu Y, Yu Z. Galectin-3 mediates the pulmonary arterial hypertension-induced right ventricular remodeling through interacting with NADPH oxidase 4. *J Am Soc Hypertens* 2017; **11**: 275-289. e2 [PMID: 28431936 DOI: 10.1016/j.jash.2017.03.008]

144 **Sharma UC**, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, André S, Crijns HJ, Gabius HJ, Maessen J, Pinto YM. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 2004; **110**: 3121-3128 [PMID: 15520318 DOI: 10.1161/01.CIR.0000147181.65298.4D]

145 **Yu L**, Ruifrok WP, Meissner M, Bos EM, van Goor H, Sanjabi B, van der Harst P, Pitt B, Goldstein IJ, Koerts JA, van Veldhuisen DJ, Bank RA, van Gilst WH, Silljé HH, de Boer RA. Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. *Circ Heart Fail* 2013; **6**: 107-117 [PMID: 23230309 DOI: 10.1161/CIRCHEARTFAILURE.112.971168]

146 **Ho JE**, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG, Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol* 2012; **60**: 1249-1256 [PMID: 22939561 DOI: 10.1016/j.jacc.2012.04.053]

147 **Ueland T**, Aukrust P, Broch K, Aakhus S, Skårdal R, Muntendam P, Gullestad L. Galectin-3 in heart failure: high levels are associated with all-cause mortality. *Int J Cardiol* 2011; **150**: 361-364 [PMID: 21641051 DOI: 10.1016/j.ijcard.2011.05.081]

148 **Sharma U**, Rhaleb NE, Pokharel S, Harding P, Rasoul S, Peng H, Carretero OA. Novel anti-inflammatory mechanisms of N-Acetyl-Ser-Asp-Lys-Pro in hypertension-induced target organ damage. *Am J Physiol Heart Circ Physiol* 2008; **294**: H1226-H1232 [PMID: 18178715 DOI: 10.1152/ajpheart.00305.2007]

149 **Keng BMH**, Gao F, Ewe SH, Tan RS, Teo LLY, Xie BQ, Koh WP, Koh AS. Galectin-3 as a candidate upstream biomarker for quantifying risks of myocardial ageing. *ESC Heart Fail* 2019; **6**:

1068-1076 [PMID: 31392851 DOI: 10.1002/ehf2.12495]
150 **Vora A**, de Lemos JA, Ayers C, Grodin JL, Lingvay I. Association of Galectin-3 With Diabetes Mellitus in the Dallas Heart Study. *J Clin Endocrinol Metab* 2019; **104**: 4449-4458 [PMID: 31162551 DOI: 10.1210/jc.2019-00398]
151 **Gopal DM**, Ayalon N, Wang YC, Siwik D, Sverdlov A, Donohue C, Perez A, Downing J, Apovian C, Silva V, Panagia M, Kolachalama V, Ho JE, Liang CS, Gokce N, Colucci WS. Galectin-3 Is Associated With Stage B Metabolic Heart Disease and Pulmonary Hypertension in Young Obese Patients. *J Am Heart Assoc* 2019; **8**: e011100 [PMID: 30929550 DOI: 10.1161/JAHA.118.011100]
152 **Holmager P**, Egstrup M, Gustafsson I, Schou M, Dahl JS, Rasmussen LM, Møller JE, Tuxen C, Faber J, Kistorp C. Galectin-3 and fibulin-1 in systolic heart failure - relation to glucose metabolism and left ventricular contractile reserve. *BMC Cardiovasc Disord* 2017; **17**: 22 [PMID: 28068900 DOI: 10.1186/s12872-016-0437-6]
153 **Flores-Ramírez R**, Azpiri-López JR, González-González JG, Ordaz-Farías A, González-Carrillo LE, Carrizales-Sepúlveda EF, Vera-Pineda R. Global longitudinal strain as a biomarker in diabetic cardiomyopathy. A comparative study with Gal-3 in patients with preserved ejection fraction. *Arch Cardiol Mex* 2017; **87**: 278-285 [PMID: 27389532 DOI: 10.1016/j.acmx.2016.06.002]
154 **Yingchoncharoen T**, Agarwal S, Popović ZB, Marwick TH. Normal ranges of left ventricular strain: a meta-analysis. *J Am Soc Echocardiogr* 2013; **26**: 185-191 [PMID: 23218891 DOI: 10.1016/j.echo.2012.10.008]
155 **Altara R**, Ghali R, Mallat Z, Cataliotti A, Booz GW, Zouein FA. Conflicting vascular and metabolic impact of the IL-33/sST2 axis. *Cardiovasc Res* 2018; **114**: 1578-1594 [PMID: 29982301 DOI: 10.1093/cvr/cvy166]
156 **Schmitz J**, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals *via* the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; **23**: 479-490 [PMID: 16286016 DOI: 10.1016/j.immuni.2005.09.015]
157 **Pascual-Figal DA**, Januzzi JL. The biology of ST2: the International ST2 Consensus Panel. *Am J Cardiol* 2015; **115**: 3B-7B [PMID: 25665766 DOI: 10.1016/j.amjcard.2015.01.034]
158 **Pusceddu I**, Dieplinger B, Mueller T. ST2 and the ST2/IL-33 signalling pathway-biochemistry and pathophysiology in animal models and humans. *Clin Chim Acta* 2019; **495**: 493-500 [PMID: 31136737 DOI: 10.1016/j.cca.2019.05.023]
159 **Benoit JL**, Hicks CW, Engineer RS, Hart KW, Lindsell CJ, Peacock WF. ST2 in emergency department patients with noncardiac dyspnea. *Acad Emerg Med* 2013; **20**: 1207-1210 [PMID: 24165288 DOI: 10.1111/acem.12250]
160 **Haider T**, Simader E, Hacker P, Ankersmit HJ, Heinz T, Hajdu S, Negrin LL. Increased serum concentrations of soluble ST2 are associated with pulmonary complications and mortality in polytraumatized patients. *Clin Chem Lab Med* 2018; **56**: 810-817 [PMID: 29341938 DOI: 10.1515/cclm-2017-0762]
161 **Vocca L**, Di Sano C, Uasuf CG, Sala A, Riccobono L, Gangemi S, Albano GD, Bonanno A, Gagliardo R, Profita M. IL-33/ST2 axis controls Th2/IL-31 and Th17 immune response in allergic airway diseases. *Immunobiology* 2015; **220**: 954-963 [PMID: 25747940 DOI: 10.1016/j.imbio.2015.02.005]
162 **Ruiz-Castilla M**, Bosacoma P, Dos Santos B, Baena J, Guilabert P, Marin-Corral J, Masclans JR, Roca O, Barret JP. Soluble Suppression Of Tumorigenicity-2 Predicts Hospital Mortality in Burn Patients: An Observational Prospective Cohort Pilot Study. *Shock* 2019; **51**: 194-199 [PMID: 29642231 DOI: 10.1097/SHK.0000000000001155]
163 **Yancy CW**, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, Hollenberg SM, Lindenfeld J, Masoudi FA, McBride PE, Peterson PN, Stevenson LW, Westlake C. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. *Circulation* 2017; **136**: e137-e161 [PMID: 28455343 DOI: 10.1161/CIR.0000000000000509]
164 **Fousteris E**, Melidonis A, Panoutsopoulos G, Tzirogiannis K, Foussas S, Theodosis-Georgilas A, Tzerefos S, Matsagos S, Boutati E, Economopoulos T, Dimitriadis G, Raptis S. Toll/interleukin-1 receptor member ST2 exhibits higher soluble levels in type 2 diabetes, especially when accompanied with left ventricular diastolic dysfunction. *Cardiovasc Diabetol* 2011; **10**: 101 [PMID: 22104207 DOI: 10.1186/1475-2840-10-101]
165 **Kiencke S**, Handschin R, von Dahlen R, Muser J, Brunner-Larocca HP, Schumann J, Felix B, Berneis K, Rickenbacher P. Pre-clinical diabetic cardiomyopathy: prevalence, screening, and outcome. *Eur J Heart Fail* 2010; **12**: 951-957 [PMID: 20581103 DOI: 10.1093/eurjhf/hfq110]
166 **Devaux Y**, Zangrando J, Schroen B, Creemers EE, Pedrazzini T, Chang CP, Dorn GW 2nd, Thum T, Heymans S; Cardiolinc network. Long noncoding RNAs in cardiac development and ageing. *Nat Rev Cardiol* 2015; **12**: 415-425 [PMID: 25855606 DOI: 10.1038/nrcardio.2015.55]
167 **Thum T**, Condorelli G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. *Circ Res* 2015; **116**: 751-762 [PMID: 25677521 DOI: 10.1161/CIRCRESAHA.116.303549]
168 **Uchida S**, Dimmeler S. Long noncoding RNAs in cardiovascular diseases. *Circ Res* 2015; **116**: 737-750 [PMID: 25677520 DOI: 10.1161/CIRCRESAHA.116.302521]
169 **Kumarswamy R**, Bauters C, Volkmann I, Maury F, Fetisch J, Holzmann A, Lemesle G, de Groote

P, Pinet F, Thum T. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. *Circ Res* 2014; **114**: 1569-1575 [PMID: 24663402 DOI: 10.1161/CIRCRESAHA.114.303915]

170 **Vausort M**, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. *Circ Res* 2014; **115**: 668-677 [PMID: 25035150 DOI: 10.1161/CIRCRESAHA.115.303836]

171 **Liu JY**, Yao J, Li XM, Song YC, Wang XQ, Li YJ, Yan B, Jiang Q. Pathogenic role of lncRNA-MALAT1 in endothelial cell dysfunction in diabetes mellitus. *Cell Death Dis* 2014; **5**: e1506 [PMID: 25356875 DOI: 10.1038/cddis.2014.466]

172 **Yan B**, Yao J, Liu JY, Li XM, Wang XQ, Li YJ, Tao ZF, Song YC, Chen Q, Jiang Q. lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA. *Circ Res* 2015; **116**: 1143-1156 [PMID: 25587098 DOI: 10.1161/CIRCRESAHA.116.305510]

173 **Carter G**, Miladinovic B, Patel AA, Deland L, Mastorides S, Patel NA. Circulating long noncoding RNA GAS5 Levels are correlated to prevalence of type 2 diabetes mellitus. *BBA Clin* 2015; **4**: 102-107 [PMID: 26675493 DOI: 10.1016/j.bbacli.2015.09.001]

174 **de Gonzalo-Calvo D**, Kenneweg F, Bang C, Toro R, van der Meer RW, Rijzewijk LJ, Smit JW, Lamb HJ, Llorente-Cortes V, Thum T. Circulating long-non coding RNAs as biomarkers of left ventricular diastolic function and remodelling in patients with well-controlled type 2 diabetes. *Sci Rep* 2016; **6**: 37354 [PMID: 27874027 DOI: 10.1038/srep37354]

175 **Ambros V**. The functions of animal microRNAs. *Nature* 2004; **431**: 350-355 [PMID: 15372042 DOI: 10.1038/nature02871]

176 **Filipowicz W**, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008; **9**: 102-114 [PMID: 18197166 DOI: 10.1038/nrg2290]

177 **Ong SG**, Wu JC. Exosomes as potential alternatives to stem cell therapy in mediating cardiac regeneration. *Circ Res* 2015; **117**: 7-9 [PMID: 26089361 DOI: 10.1161/CIRCRESAHA.115.306593]

178 **Wong LL**, Wang J, Liew OW, Richards AM, Chen YT. MicroRNA and Heart Failure. *Int J Mol Sci* 2016; **17**: 502 [PMID: 27058529 DOI: 10.3390/ijms17040502]

179 **LeBoeuf RA**, Kerckaert GA, Aardema MJ, Gibson DP. Multistage neoplastic transformation of Syrian hamster embryo cells cultured at pH 6.70. *Cancer Res* 1990; **50**: 3722-3729 [PMID: 2340519 DOI: 10.1371/journal.pone.0055672]

180 **Vasudevan S**. Posttranscriptional upregulation by microRNAs. *Wiley Interdiscip Rev RNA* 2012; **3**: 311-330 [PMID: 22072587 DOI: 10.1002/wrna.121]

181 **Voelter-Mahlknecht S**. Epigenetic associations in relation to cardiovascular prevention and therapeutics. *Clin Epigenetics* 2016; **8**: 4 [PMID: 26779291 DOI: 10.1186/s13148-016-0170-0]

182 **Zhou Q**, Lv D, Chen P, Xu T, Fu S, Li J, Bei Y. MicroRNAs in diabetic cardiomyopathy and clinical perspectives. *Front Genet* 2014; **5**: 185 [PMID: 25009554 DOI: 10.3389/fgene.2014.00185]

183 **Thrainsdottir IS**, Aspelund T, Thorgeirsson G, Gudnason V, Hardarson T, Malmberg K, Sigurdsson G, Rydén L. The association between glucose abnormalities and heart failure in the population-based Reykjavik study. *Diabetes Care* 2005; **28**: 612-616 [PMID: 15735197 DOI: 10.2337/diacare.28.3.612]

184 **Feng B**, Chen S, George B, Feng Q, Chakrabarti S. miR133a regulates cardiomyocyte hypertrophy in diabetes. *Diabetes Metab Res Rev* 2010; **26**: 40-49 [PMID: 20013939 DOI: 10.1002/dmrr.1054]

185 **Duisters RF**, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, Herias V, van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE. miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res* 2009; **104**: 170-178, 6p following 178 [PMID: 19096030 DOI: 10.1161/CIRCRESAHA.108.182535]

186 **León LE**, Rani S, Fernandez M, Larico M, Calligaris SD. Subclinical Detection of Diabetic Cardiomyopathy with MicroRNAs: Challenges and Perspectives. *J Diabetes Res* 2016; **2016**: 6143129 [PMID: 26770988 DOI: 10.1155/2016/6143129]

187 **Zhao F**, Li B, Wei YZ, Zhou B, Wang H, Chen M, Gan XD, Wang ZH, Xiong SX. MicroRNA-34a regulates high glucose-induced apoptosis in H9c2 cardiomyocytes. *J Huazhong Univ Sci Technolog Med Sci* 2013; **33**: 834-839 [PMID: 24337844 DOI: 10.1007/s11596-013-1207-7]

188 **Li X**, Du N, Zhang Q, Li J, Chen X, Liu X, Hu Y, Qin W, Shen N, Xu C, Fang Z, Wei Y, Wang R, Du Z, Zhang Y, Lu Y. MicroRNA-30d regulates cardiomyocyte pyroptosis by directly targeting foxo3a in diabetic cardiomyopathy. *Cell Death Dis* 2014; **5**: e1479 [PMID: 25341033 DOI: 10.1038/cddis.2014.430]

189 **Enomoto M**, Ishizu T, Seo Y, Kameda Y, Suzuki H, Shimano H, Kawakami Y, Aonuma K. Myocardial dysfunction identified by three-dimensional speckle tracking echocardiography in type 2 diabetes patients relates to complications of microangiopathy. *J Cardiol* 2016; **68**: 282-287 [PMID: 27146366 DOI: 10.1016/j.jjcc.2016.03.007]

190 **Ernande L**, Bergerot C, Girerd N, Thibault H, Davidsen ES, Gautier Pignon-Blanc P, Amaz C, Croisille P, De Buyzere ML, Rietzschel ER, Gillebert TC, Moulin P, Altman M, Derumeaux G. Longitudinal myocardial strain alteration is associated with left ventricular remodeling in asymptomatic patients with type 2 diabetes mellitus. *J Am Soc Echocardiogr* 2014; **27**: 479-488 [PMID: 24508363 DOI: 10.1016/j.echo.2014.01.001]

191 **Liu JH**, Chen Y, Yuen M, Zhen Z, Chan CW, Lam KS, Tse HF, Yiu KH. Incremental prognostic value of global longitudinal strain in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol*

2016; **15**: 22 [PMID: 26842466 DOI: 10.1186/s12933-016-0333-5]

192 **Holland DJ**, Marwick TH, Haluska BA, Leano R, Hordern MD, Hare JL, Fang ZY, Prins JB, Stanton T. Subclinical LV dysfunction and 10-year outcomes in type 2 diabetes mellitus. *Heart* 2015; **101**: 1061-1066 [PMID: 25935767 DOI: 10.1136/heartjnl-2014-307391]

193 **Sasso FC**, Rambaldi PF, Carbonara O, Nasti R, Torella M, Rotondo A, Torella R, Mansi L. Perspectives of nuclear diagnostic imaging in diabetic cardiomyopathy. *Nutr Metab Cardiovasc Dis* 2010; **20**: 208-216 [PMID: 19939648 DOI: 10.1016/j.numecd.2009.08.013]

194 **Tillquist MN**, Maddox TM. Update on diabetic cardiomyopathy: inches forward, miles to go. *Curr Diab Rep* 2012; **12**: 305-313 [PMID: 22528596 DOI: 10.1007/s11892-012-0274-7]

195 **Sengeløv M**, Jørgensen PG, Jensen JS, Bruun NE, Olsen FJ, Fritz-Hansen T, Nochioka K, Biering-Sørensen T. Global Longitudinal Strain Is a Superior Predictor of All-Cause Mortality in Heart Failure With Reduced Ejection Fraction. *JACC Cardiovasc Imaging* 2015; **8**: 1351-1359 [PMID: 26577264 DOI: 10.1016/j.jcmg.2015.07.013]

196 **Liu X**, Yang ZG, Gao Y, Xie LJ, Jiang L, Hu BY, Diao KY, Shi K, Xu HY, Shen MT, Ren Y, Guo YK. Left ventricular subclinical myocardial dysfunction in uncomplicated type 2 diabetes mellitus is associated with impaired myocardial perfusion: a contrast-enhanced cardiovascular magnetic resonance study. *Cardiovasc Diabetol* 2018; **17**: 139 [PMID: 30373588 DOI: 10.1186/s12933-018-0782-0]

197 **Liu L**, Ding W, Li R, Ye X, Zhao J, Jiang J, Meng L, Wang J, Chu S, Han X, Peng F. Plasma levels and diagnostic value of catestatin in patients with heart failure. *Peptides* 2013; **46**: 20-25 [PMID: 23702300 DOI: 10.1016/j.peptides.2013.05.003]

198 **Borovac JA**, Glavas D, Susilovic Grabovac Z, Supe Domic D, Stanisic L, D'Amario D, Kwok CS, Bozic J. Circulating sST2 and catestatin levels in patients with acute worsening of heart failure: a report from the CATSTAT-HF study. *ESC Heart Fail* 2020; **7**: 2818-2828 [PMID: 32681700 DOI: 10.1002/ehf2.12882]

199 **Ying W**, Mahata S, Bandyopadhyay GK, Zhou Z, Wollam J, Vu J, Mayoral R, Chi NW, Webster NJG, Corti A, Mahata SK. Catestatin Inhibits Obesity-Induced Macrophage Infiltration and Inflammation in the Liver and Suppresses Hepatic Glucose Production, Leading to Improved Insulin Sensitivity. *Diabetes* 2018; **67**: 841-848 [PMID: 29432123 DOI: 10.2337/db17-0788]

200 **Gallo MP**, Femminò S, Antoniotti S, Querio G, Alloatti G, Levi R. Catestatin Induces Glucose Uptake and GLUT4 Trafficking in Adult Rat Cardiomyocytes. *Biomed Res Int* 2018; **2018**: 2086109 [PMID: 30370303 DOI: 10.1155/2018/2086109]

201 **Kanamori H**, Takemura G, Goto K, Tsujimoto A, Mikami A, Ogino A, Watanabe T, Morishita K, Okada H, Kawasaki M, Seishima M, Minatoguchi S. Autophagic adaptations in diabetic cardiomyopathy differ between type 1 and type 2 diabetes. *Autophagy* 2015; **11**: 1146-1160 [PMID: 26042865 DOI: 10.1080/15548627.2015.1051295]

202 **Hölscher ME**, Bode C, Bugger H. Diabetic Cardiomyopathy: Does the Type of Diabetes Matter? *Int J Mol Sci* 2016; **17** [PMID: 27999359 DOI: 10.3390/ijms17122136]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 706-729

DOI: 10.4239/wjd.v12.i6.706

ISSN 1948-9358 (online)

REVIEW

Critical review of bone health, fracture risk and management of bone fragility in diabetes mellitus

Rajan Palui, Subhodip Pramanik, Sunetra Mondal, Sayantan Ray

ORCID number: Rajan Palui 0000-0002-2429-3595; Subhodip Pramanik 0000-0002-3196-1192; Sunetra Mondal 0000-0003-3064-466X; Sayantan Ray 0000-0002-6274-465X.

Author contributions: Palui R performed the literature search, wrote the first draft and provided intellectual input; Pramanik S and Mondal S conceptualized the work, performed a literature search, supervised the writing, provided intellectual input and critically revised the manuscript; Ray S supervised the literature search and the writing, provided intellectual input and critically revised the manuscript.

Conflict-of-interest statement: Nothing to disclose.

Rajan Palui, Department of Endocrinology, The Mission Hospital, Durgapur 713212, West Bengal, India

Subhodip Pramanik, Department of Endocrinology, Neotia Getwel Healthcare Centre, Siliguri 734010, West Bengal, India

Sunetra Mondal, Department of Endocrinology, Institute of Post Graduate Medical Education and Research (IPGMER), Kolkata 700020, West Bengal, India

Sayantan Ray, Department of Endocrinology, Medica Superspeciality Hospital and Medica Clinic, Kolkata 700099, West Bengal, India

Sayantan Ray, Department of Endocrinology, Jagannath Gupta Institute of Medical Sciences and Hospital, Kolkata 700137, West Bengal, India

Corresponding author: Sayantan Ray, MBBS, MD, Department of Endocrinology, Medica Superspeciality Hospital and Medica Clinic, 127, EM Bypass, Mukundapur, Kolkata 700099, West Bengal, India. sayantan.ray30@gmail.com

Abstract

The risk of fracture is increased in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). However, in contrast to the former, patients with T2DM usually possess higher bone mineral density. Thus, there is a considerable difference in the pathophysiological basis of poor bone health between the two types of diabetes. Impaired bone strength due to poor bone microarchitecture and low bone turnover along with increased risk of fall are among the major factors behind elevated fracture risk. Moreover, some antidiabetic medications further enhance the fragility of the bone. On the other hand, antiosteoporosis medications can affect the glucose homeostasis in these patients. It is also difficult to predict the fracture risk in these patients because conventional tools such as bone mineral density and Fracture Risk Assessment Tool score assessment can underestimate the risk. Evidence-based recommendations for risk evaluation and management of poor bone health in diabetes are sparse in the literature. With the advancement in imaging technology, newer modalities are available to evaluate the bone quality and risk assessment in patients with diabetes. The purpose of this review is to explore the patho-physiology behind poor bone health in diabetic patients. Approach to the fracture risk evaluation in both T1DM and T2DM as well as the pragmatic use and efficacy of the available treatment options have been discussed in depth.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: India

Peer-review report's scientific quality classification

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

Received: January 27, 2021
Peer-review started: January 27, 2021
First decision: March 30, 2021
Revised: April 8, 2021
Accepted: April 29, 2021
Article in press: April 29, 2021
Published online: June 15, 2021

P-Reviewer: Pastromas S
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Ma YJ

Key Words: Diabetes; Fracture risk; Bone mineral density; Microarchitecture; Antidiabetic drugs; Antiosteoporosis therapy

Core Tip: Diabetes mellitus, either type 1 or type 2, has adverse effects on bone that translate into an elevated fracture risk. Different pathophysiological mechanisms contribute to poor bone health in patients with diabetes. Diagnosis of bone fragility in diabetic patients is challenging as traditional fracture predictors underestimate fracture risk in this population, contributing to the concept that diabetes affects bone quality. While waiting for further evidence, the prevention and management of bone fragility in diabetes should include identification of patients at risk, correction of modifiable risk factors, appropriate choice of antidiabetic medications and use of antiosteoporosis drugs with proven efficacy.

Citation: Palui R, Pramanik S, Mondal S, Ray S. Critical review of bone health, fracture risk and management of bone fragility in diabetes mellitus. *World J Diabetes* 2021; 12(6): 706-729
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/706.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.706

INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing worldwide, along with diabetes-related renal and cardiovascular complications, in particular, resulting in an enormous burden on healthcare systems[1].DM adversely affects the skeleton as well, and the increased risk of fragility fractures is an important complication in diabetics [2]. Given the different pathogenic mechanisms of type 1 DM (T1DM) and type 2 DM (T2DM), they exhibit a unique relationship with bones. Recent evidence shows that both T1DM and T2DM are associated with an increased risk of fracture[3]. However, the relative contribution of low bone strength and increased incidence of falls behind the higher fracture risk in diabetic patients remains unknown. Fracture risk increases with duration of disease, poor glycemic control and the presence of vascular complications[4]. From a clinical standpoint, there are several challenges in the management too. Both bone mineral density (BMD) T-score and Fracture Risk Assessment Tool (FRAX) underestimate fracture prediction in patients with diabetes, particularly T2DM [5]. Moreover, antidiabetic medications have differential effects on bone homeostasis and fracture risk. The coexistence of DM and osteoporosis runs the risk of significant associated morbidity and mortality; it may also lead to significant debilitation. Thus, understanding their complex interaction is integral to providing optimal care for these patients. The purpose of this manuscript is to review the current knowledge of the factors and interconnected mechanisms that negatively affect several determinants of bone strength in patients with DM. In addition, keeping future perspectives in mind, the considerations regarding management in this population from the glycemic and the skeletal point of view are discussed.

EFFECT OF INSULIN AND INSULIN RESISTANCE OVER BONE

Insulin is anabolic for bones. Animal studies have clarified the complex mechanism through which insulin regulates bone turnover. Insulin exerts direct anabolic actions by activation of its cognate receptor. This insulin-like growth factor 1 (IGF-1) receptor has a crucial role in the execution of anabolic effects of insulin on osteoblasts[6]. As a result, insulin-deficient conditions like T1DM are typically associated with low levels and/or action of IGF-1. The role of amylin (cosecreted with insulin from pancreatic β-cell, thus low in T1DM) is unclear to date. Few studies have shown high serum levels of this factor to correlate with high bone mass[7]. However, further studies are needed to conclude its role in bone health in T1DM.

On the other hand, insulin resistance (IR), the most important feature of T2DM, affects bone quality directly in two ways. First, high blood glucose in circulation induces osteoblast resistance to the actions of IGF-1[8]. Second, high concentrations of advanced glycated end products (AGEs) impair the stimulatory actions of IGF-1 on osteoblasts[9]. Additionally, IR and adipose tissue dysregulation contribute to chronic low-grade inflammation, which can promote bone loss. In this population, loss of Dock 7 protein and silencing of Thy-1 expression induce higher bone resorption and increased adipogenesis, which leads to the impaired bone formation which in turn contributes to low bone mass[10]. Obesity-induced hypogonadism has also been implicated in the pathogenesis of low bone mass in IR state[11].

Mechanical loading is crucial for bone health as it stimulates the mechanosensitivity of osteoblasts through the Wnt-β-catenin pathway. It also increases the expression of Runt-related transcription factor 2 and consequently promotes osteogenesis. It inhibits dickkopf-related protein 1 and sclerostin secretion, resulting in attenuated bone resorption[12]. Some suggest skeletal loading may be compromised in consequence of a decrease in muscle strength due to decreased glucose uptake by muscles; however, this postulation is yet to be confirmed. Animal data show high-fat diet-induced obesity, achieved after 14-24 wk of high-fat diet consumption, leads to higher bone resorption, lower bone formation, poor quality of bone architecture and loss of bone strength[13]. Thus, both insulin deficiency and IR is associated with low bone mass. Nevertheless, hyperinsulinemia secondary to IR might contribute to high BMD in T2DM.

BONE HEALTH IN T1DM

Fracture risk in T1DM

The risk for fractures in patients with T1DM is three-fold higher than in the general population[14,15]. According to a large meta-analysis, the pooled relative risk in patients with T1DM compared to controls was 3.78 (95% confidence interval (CI): 2.05-6.98; $P< 0.001$) for hip fractures and 2.88 (95%CI: 1.71-4.82; $P< 0.001$) for vertebral fractures[15]. Studies suggest a stronger association of hip and vertebral fractures with T1DM (relative risk 6.3 and 6.94, respectively) compared to T2DM (relative risk 1.7 and 1.38, respectively) in both men and women[16]. The increased risk of fractures in patients with T1DM extends through the entire life span and starts 10 to 15 years earlier than nondiabetic populations[17]. The fracture risk is related to the duration of diabetes, with some studies revealing a near-linear relationship[18], and some suggest a bimodal relationship, with the highest incidence occurring within the first 2.5 years of diagnosing DM and a second peak occurring after 5 years[19]. Most studies fail to establish any association with glycemic control. However, the risk is higher in T1DM with microvascular complications.

Mechanisms of increased bone fragility in T1DM

The increased fracture risk in T1DM is not solely explained by changes in BMD. Other mechanisms involving alterations in bone quality, microarchitecture and bone turnover have been suggested. Figure 1 summarizes the pathophysiologic events leading to increased risk of fractures in T1DM.

BMD in T1DM: Studies on BMD in subjects with T1DM have reported a decrease in BMD ranging from 8% to 67% with the hip being the worst affected (approximately 37%) followed by vertebrae (approximately 22%)[16,20]. A high proportion of 25(OH)D deficiency and low IGF-1 in children and adolescents with T1DM has been found, which might contribute to low axial BMD[21]. Data regarding the age of onset of osteopenia in patients with T1DM are conflicting, as studies show low Z-scores in children and young adults, but no differences in adults with T1DM in comparison to nondiabetics. A recent meta-analysis showed a significant reduction of BMD in children with T1DM[22]. Normalization of BMD or bone size over time in patients with T1DM is seen in longitudinal studies. Poor glycemic status can adversely affect BMD during childhood and adolescence, even though dual-energy X-ray absorptiometry (DXA) may not identify osteoporotic range for BMD[23]. An inverse correlation between BMD scores with glycated hemoglobin/hemoglobin A1c (HbA1c) and the duration of diabetes has been noticed in many but not all studies, yet the association with microvascular complications have been more consistent[24-26]. Results of the trabecular bone score (TBS) in T1DM have been inconsistent. Diabetics with microvascular disease have been seen to have lower total, cortical and trabecular

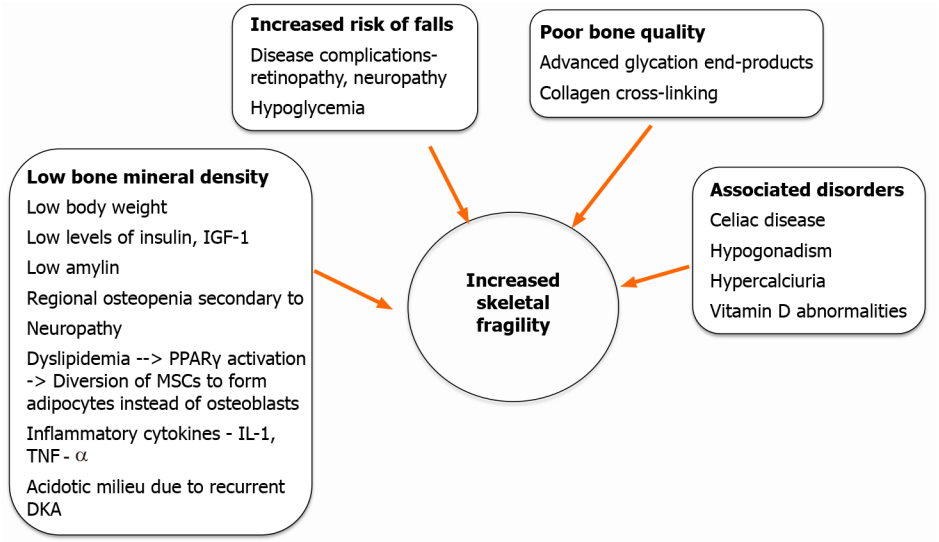

Figure 1 Mechanisms of increased bone fragility in type 1 diabetes mellitus. DKA: Diabetic ketoacidosis; IGF-1: Insulin-like growth factor; PPAR: Peroxisome proliferator-activated receptor; MSC: Mesenchymal stem cell;IL-1: Interleukin 1; TNF: Tumor necrosis factor.

volumetric BMD on high-resolution peripheral quantitative computed tomography (HRpQCT) of the radius[27].

Bone turnover in T1DM: Low levels of bone formation markers like osteocalcin (OC) have been seen in patients with T1DM. OC is of particular interest in T1DM because its effects on both insulin production and insulin sensitivity have been demonstrated. Markers of bone resorption including procollagen type1 N-terminal propeptide (P1NP) and C-terminal cross-link of collagen (CTX) are either low or unaltered in T1DM. These together hint towards T1DM as an overall, low bone turnover state [28, 29].

Data on bone histomorphometry, the gold standard for the study of bone turnover is scarce in T1DM. No differences were seen in bone formation or resorption markers in a cohort of T1DM compared to controls. However, among those with a history of fractures, reduced activation frequency and increased degree of mineralization and nonenzymatic collagen crosslinks, were observed that suggest a low turnover state[30].

Bone geometry-size and structure: T1DM predominantly affects the cortical bone structure, whereas changes to trabecular bone are less pronounced. Lower cortical thickness but with the increased cross-sectional area has been demonstrated in long bones. The overall bone size is not smaller, but they have a larger endosteal circumference likely due to an enlarged trabecular bone compartment[31]. Though there are no differences in trabecular microarchitecture from controls, patients with T1DM and concomitant microvascular disease have thinner trabeculae, a lesser number of trabeculae *per* unit volume with increased spacing in between as observed on HRpQCT and magnetic resonance imaging[32]. A recent study using HRpQCT in adolescents shows detrimental changes in tibial and radial microarchitecture and bone strength, even before changes in BMD occur. Thus, the reduction in bone strength must have been related to poor glycemic control earlier in life. This study highlights that changes in bone microarchitecture and strength in early life in those with T1DM, rather than bone density, can predict the increased risk of fracture observed in adults [33].

Alteration in bone tissue quality: Although much is unknown, some analogy can be drawn with changes in T2DM, including accumulation of AGEs, increased collagen cross-linking, altered expression of noncollagenous protein expression and occlusion of vascular channels with microvascular disease, all of which can stiffen the organic matrix and increase fragility.

Reduced bone turnover in T1DM leads to the accumulation of aging bone material, and shifts to a more carbonated bone mineral matrix, which can detrimentally influence bone tissue strength[34,35].

Nonosseous factors contributing to bone fragility: Recurrent hypoglycemic episodes, low body weight, microvascular complications especially peripheral neuropathy,

autonomic neuropathy and retinopathy can all contribute to the increased risk of falls in patients with T1DM. Additional factors like concomitant uncorrected hypothyroidism, celiac disease, hypogonadism and low IGF-1 levels can also contribute to poor muscle strength. However, data regarding the relative contribution of these factors and susceptibility to fracture risk are not yet available.

BONE HEALTH IN T2DM

Epidemiology of fracture risk in T2DM

Patients with T2DM carry less fracture risk than the T1DM category but are subject to an increased risk of overall fractures (5%-24%)[16,36-38]. The meta-analyses that evaluated the fracture risk in T2DM patients are summarized in Table 1. Among the skeletal sites, increased risk of hip fracture (8%-70%) has been reported consistently in most of the meta-analyses[14,39-42]. Young age, prolonged duration of diabetes, use of insulin[39] and Asian ethnicity[40] are the factors that have been associated with a higher risk of hip fracture in diabetic patients. However, the risk of fracture is not comparable at all the skeletal sites. Increased risk of new (incident) vertebral fracture has been reported by Koromani *et al*[43], but the same meta-analysis also reports a lesser rate of prevalent vertebral fracture in diabetic patients in comparison to controls [43]. Among the other nonvertebral fractures, significantly increased risk of the ankle [37,44,45], wrist[16,45] and arm fractures[37,44] have been reported in some but not in all the meta-analyses.

BMD in T2DM patients

In a meta-analysis[46] of 15 observational studies, BMD at different sites was compared between 3437 T2DM patients and 19139 controls. The pooled analyses showed a significant increase in BMD at hip (0.06 g/cm^2), femoral neck (0.04 g/cm^2) and spine (0.06 g/cm^2). The meta-regression analysis showed higher HbA1c, body mass index, young age and male gender to be associated with high BMD in T2DM patients[46]. In another meta-analysis[16], where pooled analysis of BMD was evaluated in both T1DM and T2DM patients, BMD was found to be significantly increased in the latter but decreased in T1DM patients. In this meta-analysis also, body mass index was a significant predictor of BMD in T2DM patients. Obesity and hyperinsulinemia could be the major reasons behind the higher BMD in type 2 diabetic patients. Even in prediabetic male patients, BMD was found to be higher at the femoral neck in a study from South Korea[47]. However, BMD was significantly less at the femoral neck among obese T2DM children at the time of diagnosis of diabetes[48]. In a study from India, no significant difference in BMD was found between T2DM and controls[49]. Though different studies show inconsistent results, most have reported higher BMD in adult T2DM patients than in controls. The risk of increased bone fragility in T2DM patients with relatively higher BMD suggests a paradoxical phenomenon, contrary to the findings in the general population.

Structural bone quality in T2DM

Alteration in bone microarchitecture leading to poor bone quality can be one of the major reasons behind the increased fracture risk in T2DM patients. TBS can act as a surrogate for bone microarchitecture. A meta-analysis that included 40508 individuals from 12 studies found significantly lower TBS (standardized mean difference: -0.31, 95%CI: -0.45 to -0.16) in patients with T2DM than controls[50]. Even TBS in patients with prediabetes was also significantly lower (standardized mean difference: -0.13, 95%CI: -0.23 to -0.04) than those with normal blood glucose[50]. Higher accretion of pentasodine, an AGE, had been correlated with lower TBS in patients with diabetes [51].

HRpQCT is a noninvasive technique, apart from TBS, that has been used to evaluate bone architecture. In a study of elderly female diabetic patients, cortical porosity was higher at the radius (P<0.05) and tibia leading to a decrease in compressive biomechanical properties[52]. In another study, postmenopausal diabetic patients with fragility fracture had higher endocortical bone surface, intracortical pore volume and greater relative porosity at the distal tibia and ultra-distal radius than those without fracture [53].

Diabetic patients with microvascular disease had inferior cortical bone quality than those without microvascular disease[54]. Diabetes-related vascular changes (cortical microangiopathy) had been postulated as the reason behind the poor cortical bone

Table 1 Summary of meta-analyses evaluating risk of fracture in patients with type 2 diabetes mellitus

Ref.	Fracture site	Risk effect (95%CI)	*P* value	Risk factors (site)
Vilaca *et al*[39], 2020	Hip	RR 1.33 (1.19-1.49)	S	Younger age, female gender, insulin use, longer duration of diabetes (hip)
	Nonvertebral	RR 1.19 (1.11-1.28)	S	
Koromani *et al*[43], 2020	Vertebral (incident)	OR 1.35(1.27-1.44)	S	
	Vertebral (prevalent)	OR 0.84 (0.74-0.95)	S	
Wang *et al*[36], 2019	All	RR 1.22 (1.13-1.31)	S	
	Hip	RR 1.27 (1.16-1.39)	S	
	Distal forearm	RR 0.97 (0.66-1.09)	NS	
	Upper arm	RR 1.54 (1.19-1.99)	S	
	Ankle	RR 1.15 (1.01-1.31)	S	
	Vertebrae	RR 1.74 (0.96-3.16)	NS	
Liu *et al*[44], 2018	Limb	RR 1.18 (1.02-1.35)	S	Female gender (leg/ankle)
	Leg/Ankle	RR 1.80 (1.13-2.87)	S	
	Humerus	RR 1.27 (0.60-2.68)	NS	
	Wrist/hand/foot	RR 1.26 (0.94-1.71)	NS	
	Forearm	RR 0.98 (0.78-1.23)	NS	
Vilaca *et al*[45], 2019[1]	Ankle	RR 1.30 (1.15-1.48)	S	
	Wrist	RR 0.85 (0.77-0.95)	S	
Moayeri *et al*[37], 2017	All	RR 1.05 (1.04-1.06)	S	Older age, male gender, duration of diabetes. Insulin use, Corticosteroid use (overall)
	Hip	RR 1.20 (1.17-1.23)	S	
	Vertebral	RR 1.16 (1.05-1.28)	S	
	Foot	RR 1.37 (1.21-1.54)	S	
	Wrist	RR 0.98 (0.88-1.07)	NS	
	Proximal humerus	RR 1.09 (0.86-1.31)	NS	
	Ankle	RR 1.13 (0.95-1.32)	NS	
Jia *et al*[38], 2017[2]	All	IRR 1.23 (1.12-1.35)	S	
	Hip	IRR 1.08 (1.02-1.15)	S	
	Vertebrae	IRR 1.21 (0.98-1.48)	NS	
Ni and Fan[42], 2017	All LBMF	RR 1.24 (1.09-1.41)	S	Female gender
Dytfeld and Michalak[40], 2017[3]	Hip	OR 1.30 (1.07-1.57)	S	Cohort studies, Studies conducted in Asia (hip)
	Vertebral	OR 1.13 (0.94-1.37)	NS	
Fan *et al*[41], 2016	Hip	RR 1.34 (1.19-1.51)	S	
Vestergaard[16], 2007	Hip	RR 1.38 (1.25-1.53)	S	
	Wrist	RR 1.19 (1.01-1.41)	S	
	Vertebrae	RR 0.93 (0.63-1.37)	NS	
	All	RR 0.96 (0.57-1.61)	NS	
Janghorbani *et al*[14], 2007	Hip	RR 1.7 (1.3-2.2)	S	

[1]Study included both type 1 and type 2 diabetes.
[2]Low energy fractures.
[3]Low energy fractures in postmenopausal women. CI: Confidence interval; IRR: Incidence rate ratio; LBMF: Low bone mass-related fractures; NS: Not significant; OR: Odds ratio; RR: Relative risk; S: Statistically significant.

quality in diabetic patients with a fracture[55]. Moreover, cortical porosity was significantly higher in T2DM patients with peripheral vascular disease in comparison to controls, and cortical porosity was inversely correlated with transcutaneous oxygen tension[56]. On the other hand, in the Maastricht Study[57], T2DM patients with HbA1c <7% have superior cortical bone quality than those with poor glycemic control, but no significant relation was found with the microvascular disease.

To summarize, change in bone microarchitecture as evidenced by poor cortical bone quality in T2DM patients can explain to some extent the paradox of increased bone fragility despite preserved BMD in these patients. Bone material strength index (BMSi) as calculated by *in vivo* bone microindentation acts as a surrogate marker of bone strength. Reduced BMSi has been reported in T2DM patients in comparison to nondiabetic controls[58,59]. Adiposity is related to decreased BMSi and increased cortical porosity in T2DM patients[60].

Bone turnover in T2DM

The studies that evaluated bone turnover markers (BTMs) in diabetic patients mostly identified diabetes as a low turnover disease. A meta-analysis that included 22 studies comprising of both T1DM and T2DM patients reported lower levels of both bone resorption (urinary N-terminal cross-linked telopeptide of type-I collagen) and formation (OC) markers[61]. However, subgroup analysis of T2DM patients showed only a trend towards lower OC levels in comparison to nondiabetic controls. In another recent meta-analysis[62], a pooled analysis showed significantly lower resorption markers (CTX and tartrate-resistant acid phosphatase) as well as formation markers (OC and P1NP) in T2DM patients. Moreover, sclerostin was found to be significantly higher in T2DM patients[62]. The elevated sclerostin level can be the link between hyperglycemia, low bone turnover and increased fracture risk in T2DM[63,64]. In a bone histomorphometry study, reduced mineralization surface, mineral apposition rate, bone formation rate and adjusted apposition rate along with a significant increase in mineralization lag time had been reported in eight (six type 2 and two type 1) diabetic patients in comparison to control[65]. In another study, significantly reduced mineralization surface, osteoblast surface and bone formation rate had been found in T2DM patients in comparison to nondiabetic control[66].

Pathophysiology of bone disease in T2DM

The increased fracture risk in T2DM is due to increased bone fragility and a greater risk of falls in these patients. Diminished vision, peripheral neuropathy, poor balance, diabetic arthropathy and hypoglycemic episodes can all increase the risk of falls in these patients. Moreover, antidiabetic medications can also increase fracture risk (discussed in next section). A brief outline of the pathophysiology of bone disease in T2DM has been illustrated in Figure 2. The detailed discussion of various pathophysiological mechanisms is beyond the scope of this review. The readers can find more detailed discussions on this topic elsewhere[2,67].

IMPACT OF DIABETES TREATMENTS ON BONE METABOLISM: CURRENT EVIDENCE

The complexity of bone changes in diabetes is made more complicated by the plethora of effects that pharmacotherapy induces. Therapeutic agents for diabetes affect bone quality and fracture risk by different mechanisms. First, they can affect bone formation and resorption at the molecular level. Second, some agents induce weight loss that can independently be associated with a reduction of bone mass. Third, agents that increase the chance of fall especially in the elderly can increase the chance of fracture, irrespective of bone quality. The effects of individual agents are discussed in Table 2 [68-87].

Overall, insulin, metformin and glucagon-like peptide 1 analogs have a beneficial effect, and pioglitazone and bariatric surgery have a negative effect on bone morphology. Agents like sulfonylureas, dipeptidyl peptidase-4 inhibitors and sodium-glucose cotransporter-2 inhibitors do not have any direct beneficial or detrimental effects on bone morphology. But the fracture outcome data with all these agents depend on concomitant weight loss and risk for hypoglycemia. To date, only pioglitazone, insulin and bariatric surgery have demonstrated an increased risk for fracture in a real-world setting. However, whether insulin actually increases the fracture risk is controversial. Insulin-treated patients on average have longer disease duration and a higher prevalence of micro-and macrovascular complications. Thus,

Table 2 The effect of diabetes therapies on skeletal parameters and fracture risk

Agents	Effect on bone metabolism	Additional effects on fracture risk	Effect on bone markers and BMD	Effect on fracture	Overall effect
Insulin	Anabolic	Increases fall risk[68]	No negative effect	Hip, peripheral and osteoporotic fracture risk is magnified[69]. A propensity matched cohort analysis demonstrated adjusted sub hazard ratio of 1.38 (95%CI: 1.06-1.80) for major fractures with insulin use as compared with nonusers[70]. Females are more prone. No increased risk with glargine use[71]	Effect on bone +ve. Fracture risk ↑
Metformin	Anabolic (*via* AMPK). Skew the mesenchymal stem cells from the adipogenic to the osteogenic arm[72] and inhibit osteoclast differentiation[73]	Reductions in oxidative stress and cell apoptosis		In a meta-analysis the use of metformin was associated with a reduced risk of fracture (RR 0.86, 95%CI: 0.75-0.99). It was mostly prescribed in the early stages of T2DM, and there was less hypoglycemia that might explain fewer fractures with metformin[74]	Effect on bone +ve. Fracture risk ↓
Sulfonylurea	Negligible effect	Increases fall risk due to hypoglycemia	Negligible effect	A recent meta-analysis including 11 studies involving 255644 individuals showed 14% increase in the risk of developing fracture[75]. Most of the fractures were attributable to increased fall due to hypoglycemia[76]	Effect on bone-neutral. Fracture risk ↔/↑
Pioglitazone	Proadipogenic. Inhibits osteoblast differentiation. Inhibits osteoclast differentiation[77]	None	The bone resorption marker(CTX) was elevated, while indicators of bone formation were reduced[78]. It was also associated with significant reduction in BMD among women at the lumbar spine as well in femoral neck.	An updated meta-analysis including 24544 participants from 22 RCTS showed significantly increased incidence of fracture was found in women (OR=1.94; 95%CI: 1.60-2.35; P<0.001), but not in men (OR=1.02; 95%CI: 0.83-1.27; P=0.83). The fracture risk was independent of age, and there was no clear association with duration of TZD exposure[79]	Effect on bone -ve. Fracture risk ↑
DPP-4 inhibitors	Preclinical studies demonstrated antiresorptive evidence [80]	None	None	The overall risk of fracture did not differ between patients exposed to DPP-4 inhibitors and controls (RR, 0.95; 95%CI: 0.83-1.10; P = 0.50) in a meta-analysis including 62 RCTs[81]	Effect on bone-neutral. Fracture risk ↔
GLP-1 Analogues	Pro-osteoblast. Suppress sclerostin and increase osteocalcin[82]	By virtue of weight loss, they are supposed to cause a decrease in BMD	BMD did not significantly change after exenatide-induced weight loss (-3.5 ± 0.9 kg); suggesting that exenatide treatment attenuated BMD decrements after weight loss[83]	The Bayesian network meta-analysis suggested that GLP-1 RAs had a decreased bone fracture risk compared to other antihyperglycemic drugs, and exenatide is the safest agent with regard to the risk of fracture[84]	Effect on bone +ve. Fracture risk ↔
SGLT-2 inhibitors	Preclinical data are conflicting	Weight loss causes BMD loss. Increased PTH due to phosphate reabsorption	A randomized controlled study (104 wk) found that canagliflozin induced reductions in hip BMD (−1.2% relative to placebo) [85]	A recent meta-analysis including 30 RCTs demonstrated that the incidence of bone fractures was not significantly different between patients taking SGLT2 inhibitors and placebo[86]	Effect on bone ↔. Fracture risk ↔
Metabolic surgery	No direct effect. Mechanical unloading, nutritional deficiencies and hormonal changes are catabolic to bone	Massive weight loss causes a reduction of BMD. The severity of bone outcomes seems to be related to the degree of malabsorption varies depending on different procedures	Patients undergoing gastric bypass surgery, BMD was 5%-7% lower at the spine and 6%–10% lower at the hip compared with nonsurgical controls, as assessed by QCT and dual-energy X-ray absorptiometry[87]	In a large database from the United Kingdom. RYGB is associated with a 43% increased risk of nonvertebral fracture compared with AGB, with risk increasing >2 yr after surgery. The risk was highest after 5 yr of surgery (HR 3.91)[87]	Effect on bone -ve. Fracture risk ↑

AGB: Adjustable gastric banding; AMPK: AMP-activated protein kinas; BMD: Bone mineral density; CI: Confidence interval; CTX: C-terminal cross-linked telopeptide; DPP-4: Dipeptidyl-peptidase 4; GLP-1: Glucagon -like peptide-1; HR: Hazard ratio; OR: Odds ratio; PTH: Parathormone; QCT: Quantitative computed tomography; RCT: Randomized controlled trial; RYGB: Roux-en-Y gastric bypass; RR: Relative risk; SGLT-2: Sodium-glucose cotransporter 2; T2DM: Type 2 diabetes mellitus; TZD: Thiazolidinedione; ↑: Increase; ↓: Decrease; ↔: Unchanged; +ve: Positive; -ve: Negative.

insulin use may just be a surrogate for severity or duration of T2DM, risk of hypoglycemia, presence of complications or increased risk of fall, which may explain the increased fracture risk in patients with T2DM. However, given there is a paucity of evidence of fracture outcome data from randomized controlled trials (RCTs) as a primary outcome, the conclusions reached herein are subject to change with additional

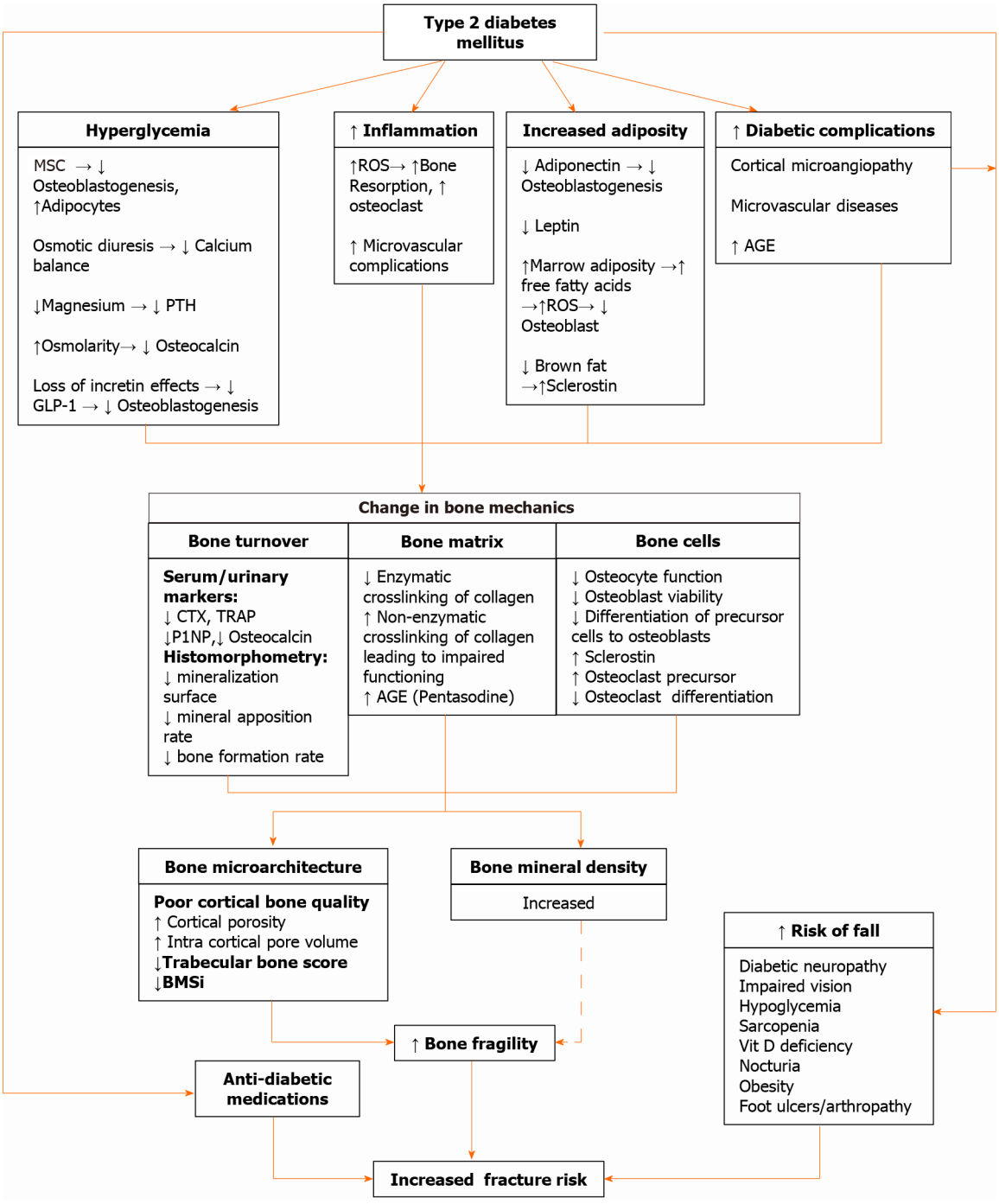

Figure 2 Mechanisms underlying bone fragility in type 2 diabetes mellitus. AGE: Advanced glycated end product; BMSi: Bone material strength index; CTX: C-terminal cross-linked telopeptide; GLP-1: Glucagon-like peptide-1; MSC: Mesenchymal stem cells; P1NP: Procollagen type 1 N-terminal propeptide; PTH: Parathyroid hormone; ROS: Reactive oxygen species; TRAP: Tartrate-resistant acid phosphatase.

future evidence.

PREDICTION OF FRACTURE RISK IN INDIVIDUALS WITH DIABETES: AN EMERGING CHALLENGE

Given the increasing number of patients with diabetes and consequently increasing the population-attributable risk of fracture, it is imperative to find out predictors of fracture. Even with increased fracture risk in diabetes, risk stratification of patients with diabetes is still lacking.

Clinical and radiologic assessment

The association between standard clinical risk factors (CRFs) for osteoporotic fractures as well as incident fractures is comparable in individuals with and without diabetes [88]. Nevertheless, other factors specific to the diabetic population need to be considered. For the duration of diabetes, studies have shown positive associations with fracture risk[89]. Poor glycemic control may impact differently on fracture risk depending on the type of diabetes. In some studies, a higher risk of fracture was observed in the presence of chronic complications of diabetes[16,90]. However, the impact of diabetic complications on fracture risk is debatable. In patients with diabetes, a history of fall is of particular importance.T2DM patients with fractures have more frequent episodes of fall and are more likely to be affected by peripheral neuropathy and reduced physical performance[91]. Vitamin D deficiency is also more common in patients with diabetes, and it is generally accepted that vitamin D-deficient subjects are at greater risk of fractures, but specific data on vitamin D-deficient patients with diabetes are not available[92].

In day-to-day practice, fracture risk is usually determined by measuring BMD (at the lumbar spine and the proximal femur) and by CRF assessment. These well-established RFs are part of a questionnaire-based FRAX released in 2008[93]. In general, BMD measured by DXA is regarded as the gold standard for bone health assessment in clinical practice. However, the estimated fracture probabilities by the BMD T-score and FRAX significantly underestimate fracture risk in patients with T1DM and T2DM[3,5]. This situation poses considerable challenges for the primary prevention of fragility fractures in these patients.

Risk assessment modalities

The bone status and fracture risk in diabetic patients may be evaluated by different approaches: BMD, CRFs, fracture probability, bone microarchitecture and bone strength.

BMD, CRFs, fracture probability: Studies have consistently demonstrated lower BMD in patients with T1DM compared to subjects without diabetes[16] and higher BMD in patients with T2DM. Importantly, for patients with both T1DM and T2DM, the BMD T-score underestimates the fracture risk[5,16]. Schwartz *et al*[5] showed that a T-score in a diabetic woman that is associated with risk of hip fracture corresponds to a T-score of approximately 0.5 units lower in a nondiabetic woman[5]. Though BMD underestimates the risk of fracture, it stratifies the risk in elderly patients with T2DM [94].

The FRAX algorithm allows for calculations of the 10year probability of fracture. The assessment is based on CRFs and the hip BMD T-score and permits for the incorporation of secondary osteoporosis for example in T1DM but not in T2DM. One prospective study found that the FRAX algorithm underestimated fracture risk in patients with T2DM[5], and a retrospective cohort study showed that FRAX underestimated the risk of hip fracture and major osteoporotic fracture in a group of combined T1DM and T2DM patients[3].

Overall, neither BMD T-score nor the FRAX tool provides a satisfactory fracture risk evaluation for patients with diabetes, and additional considerations on this topic are described in the next section.

Bone microarchitecture and bone quality: HRpQCT can be used to image and quantify volumetric BMD and bone microarchitecture including cortical porosity at a low radiation dose. Further, the estimated bone strength and failure load can be calculated. An association between high cortical porosity and T2DM was first described by Burghardt and others[52,53,95]. As determined by finite element analysis, pathologic cortical microarchitecture translated into major deficits in stiffness, failure load and cortical load fraction[52]. Recently, the Framingham Study found that T2DM patients had lesser cortical volumetric BMD, higher cortical porosity and smaller tibial cross-sectional area, independent of age, sex, weight and height[96]. Although the HRpQCT data is promising and could be a better fracture risk predictor than DXA, this research technique is unlikely to become widely available for routine clinical use.

A newer approach for the assessment of bone quality is bone indentation. Some studies using tibial outer cortex microindentation have shown that the estimated BMSi is reduced in T2DM compared to controls[59,97]. Moreover, AGE accumulation is negatively related to BMSi[97]. Nevertheless, its wide use as a clinical tool is restricted because of the invasive procedure. Taken together, available data points towards deficits in the cortical compartment and lesser resistance to the indentation in patients with diabetes.

The TBS is a parameter that reveals bone microarchitecture through analysis of DXA image pixel gray-level variations. Leslie *et al*[98] evaluated 2356 diabetic women (both T1DM and T2DM) and 27051 women without diabetes and revealed lower TBS in diabetic patients in comparison to controls in spite of higher lumbar spine and hip BMD in patients with diabetes. Current studies suggest the potential of TBS in fracture risk prediction for diabetic patients[99-101]. Clinical studies directly comparing differences in TBS between T1DM and T2DM are scarce. To summarize, because TBS is DXA based, it can be accessed without the need of new equipment, and TBS is more helpful for predicting fracture risk when combined with BMD. However, there is a lack of evidence demonstrating how post-treatment TBS improvement can decrease fracture risk[102].

Histomorphometry and BTMs: Studies in rodent models have found a reduced rate of bone turnover, worse microstructure, and lower strength in T1DM and T2DM. However, as the bone biopsy is an invasive test, only a small number of clinical studies have investigated the bone quality of patients using bone histomorphometry[102]. Moreover, results are inconsistent among different studies. A recent paper has shown that premenopausal women with T2DM have low bone turnover rates compared to healthy controls, and histomorphometry parameters are influenced by disease control and the presence of chronic complications[103]. Additional high-quality studies are necessary to determine the histologic changes of diabetic bone.

BTMs have been extensively investigated in patients with DM. A recent meta-analysis on levels of circulating BTMs in children and adolescents withT1DM reported reduced levels of OC compared to subjects without diabetes, while data were not conclusive for CTX and P1NP[104]. Another meta-analysis evaluating BTMs in both T1DM and T2DM subjects showed increased levels of alkaline phosphate in diabetic patients and decreased OC, CTX, and 25 (OH) vitamin D levels compared to controls [61]. Neither P1NP, N-terminal propeptide type1 collagen, deoxypyridinoline, bone-specific alkaline phosphatase nor parathyroid hormone (PTH) differed significantly from controls. This meta-analysis also reported considerable heterogeneity between the studies. Newer evidence suggested BTMs are decreased as CTX, OC, P1NP, u-N-terminal propeptide type 1 collagen and PTH were lower in T2DM than controls[105-107]. The association between BTMs and fracture has been evaluated in cross-sectional trials. CTX and sclerostin may potentially predict fractures, but longitudinal trials are required[108,109]. In general, BTMs are poorly related to fracture risk in patients with diabetes as bone marker levels differ from study to study. It should also be pointed out that nephropathy may alter bone turnover and modify fracture risk in diabetes.

BTMs seem to be lower in patients with diabetes, whereas bone-specific alkaline phosphatase is normal to higher, suggesting that the matrix becomes hypermineralized in diabetic patients[110]. This may clarify, in part, the paradox of low bone strength and increased BMD.

Evaluation of bone health in T1DM

There are no specific recommendations on BMD screening for T1DM patients. Following pediatric guidelines in children and adolescents with T1DM, osteoporosis can only be diagnosed in the presence of vertebral compression fractures or clinically significant long bone fractures (≥2 long-bone fractures up to the age of 10 years and ≥ 3 long-bone fractures up to age 19 years) with a BMD Z-score of 2.0 or lower[111]. The preferred sites for bone mineral content and areal BMD measurements in children include spine and total body less head but not the hip. However, there is a lack of normative data in children, and areal BMD requires adjustments for differences in height and bone size. The effects of height and bone size on BMD can be offset by an automated radiogrammetric measurement of cortical BMD of the second to fourth metacarpal bones using BoneXpert, expressed as a bone health index, and one study has reported significantly decreased cortical bone density using this technique in children and adolescents with T1DM[112].

The FRAX algorithm is used to estimate an individual's 10year probability of major osteoporotic fracture and hip fracture in subjects greater than 40 years of age. However, T1DM is considered as a cause of secondary osteoporosis, and therefore it increases fracture probability only if BMD is not included in the calculations[20]. A low TBS value can increase the predicted fracture probability in T1DM[113].

To date, it is not clear who should undergo a BMD assessment among T1DM patients. One single study suggested a number of risk factors for fractures in patients with T1DM, the presence of which should dictate the need for DXA scanning and further evaluation. Figure 3 provides an approach for investigating osteoporosis in T1DM.

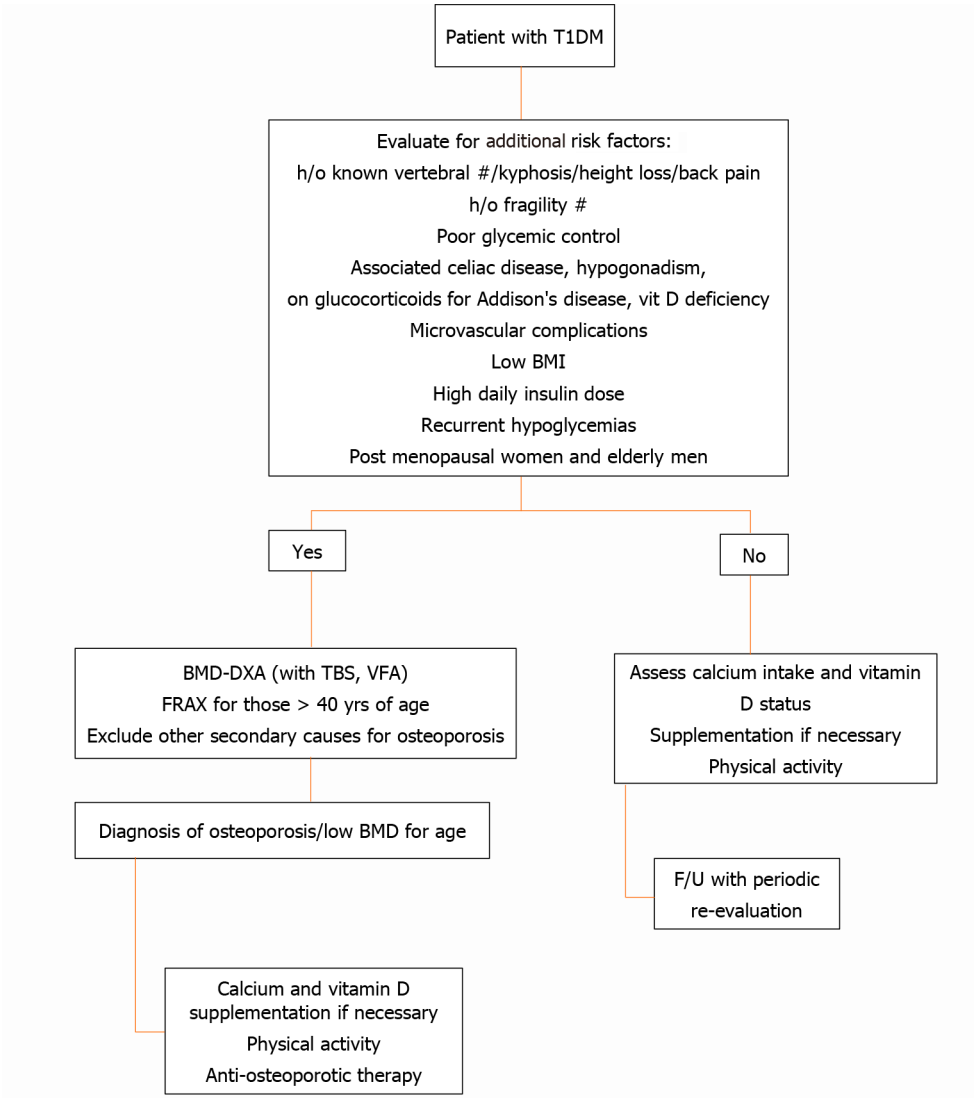

Figure 3 Algorithm for evaluation of bone health in type 1 diabetes mellitus. BMI: Body mass index; BMD-DXA: Bone mineral density by dual energy X-ray absorptiometry; F/U: Follow up; FRAX: Fracture Risk Assessment Tool; H/o: History of; T1DM: Type 1 diabetes mellitus; TBS: Trabecular bone score; VFA: Vertebral fracture assessment.

Evaluation of fracture risk in patients with T2DM-an algorithm

The identification of fracture risk in patients with T2DM remains challenging and the optimal approach has not yet been established. An algorithm for the evaluation of fracture risk in diabetic patients is proposed in Figure 4. This approach may change over time as additional evidence accumulates.

COMPREHENSIVE MANAGEMENT OF FRACTURE RISK IN PATIENTS WITH DIABETES

Nonpharmacologic management

Patients with diabetes should modify their lifestyle with optimal exercise and a balanced diet. Exercise is beneficial to improve bone strength and bone biomechanical properties[114]. Nevertheless, weight loss-associated muscle and bone loss may enhance the risk of sarcopenia and skeletal fragility. Sarcopenia and sarcopenic obesity should be prevented by sufficient protein intake and weight-bearing exercise to reduce the risk of falls and frailty[115]. Calcium and vitamin D are important in the maintenance of bone health and are included in the treatment of osteoporosis. Even though the skeletal benefits of vitamin D supplementation in diabetes have not been shown, in correspondence to the nondiabetic population, daily intake of 800 IU vitamin D may be advocated. Nevertheless, it may not be enough to attain optimal

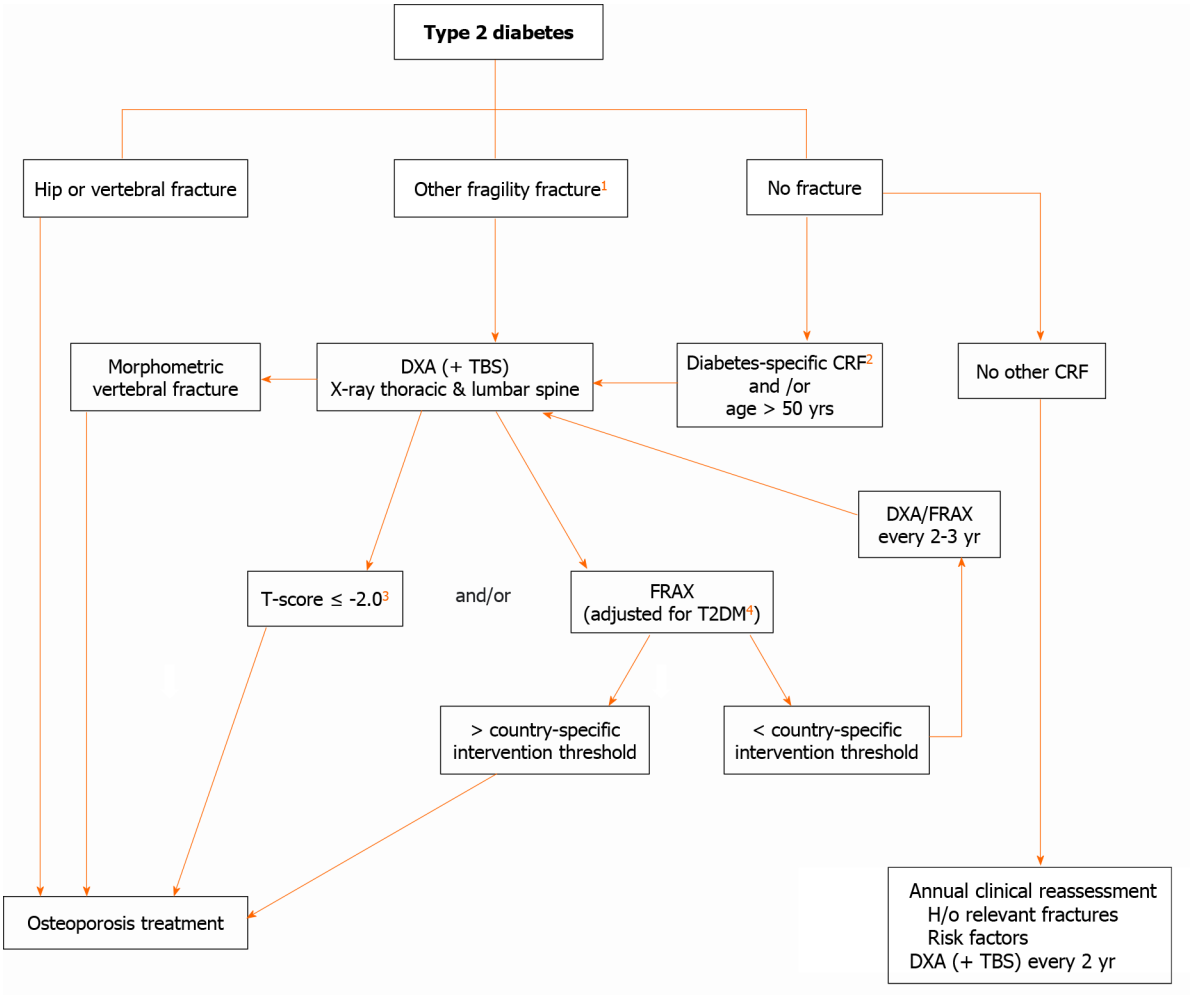

Figure 4 Evaluation of fracture risk in patients with type 2 diabetes mellitus. [1]: ≥ 1 nonvertebral nonhip fragility fracture might be required to initiate therapy; [2]: Diabetes-specific clinical risk factors (diabetes duration, antidiabetic medications,, hemoglobin A1c and microvascular complications); [3]: In diabetes, fracture risk at T-score < -2 equivalent for nondiabetes at T-score < -2.5; [4]: See text. CRF: Clinical risk factor; TBS: Trabecular bone score; DXA: Dual energy X-ray absorptiometry; T2DM: Type 2 diabetes mellitus; FRAX: Fracture Risk Assessment Tool; H/o: History of. Modified from Ferrari *et al*[123]: Ferrari SL, Abrahamsen B, Napoli N, Akesson K, Chandran M, Eastell R, El-Hajj Fuleihan G, Josse R, Kendler DL, Kraenzlin M, Suzuki A, Pierroz DD, Schwartz AV, Leslie WD; Bone and Diabetes Working Group of IOF. Diagnosis and management of bone fragility in diabetes: an emerging challenge. *Osteoporos Int* 2018; 29:2585-2596.

serum levels (30 ng/mL) in T2DM. Sufficient calcium intake (1000 mg/d) is also recommended (preferably from food sources). For children and adolescents with T1DM, calcium and vitamin D supplementation is particularly important. Screening for celiac disease and early introduction of gluten-free diet is very important in T1DM subjects. Other nonpharmacological measures like avoidance of smoking, decrease in sodium intake and limitation in alcohol consumption (< 3 units/d) remain vital.

Optimal management of diabetes in patients with concomitant osteoporosis

Glycemic control and diabetic chronic complications: Although strict glycemic control does not necessarily lessen the fracture risk[116], numerous studies have suggested that poor glycemic control enhances the fracture risk compared to control in T1DM[117,118] and T2DM[119,120]. Therefore, for diabetic patients, a smooth reduction in blood glucose level is required to avert hypoglycemia and its consequences, including fracture[121]. A strong association has been documented between diabetic complications and risk of fracture[119,122]. Peripheral neuropathy, retinopathy and any impairment of vision, predisposition to hypoglycemia, hypotension and recent history of fall should be taken into account and corrected where possible.

Choose antidiabetic drugs carefully: Medications used in the treatment of T2DM may have an impact on bone metabolism. For people with diabetes at high fracture risk, antidiabetic agents with neutral effects or even with a protective effect on bone, like

metformin, dipeptidyl peptidase-4inhibitors or glucagon-like peptide-1RA, should be preferred. Thiazolidinediones should be used with caution in elderly patients with T2DM who are at risk for fracture, especially in postmenopausal women, and the concurrent use of thiazolidinediones and sulfonylureas should be avoided in particular. Caution should be exercised when using sodium-glucose cotransporter-2 inhibitors in elderly patients with cardiovascular diseases or those taking high-dose diuretics. Insulin should be used with caution and careful measures to prevent hypoglycemia.

Indications for treatment of osteoporosis in diabetic patients

In individuals with diabetes, treatment should be considered at more favorable BMD and FRAX values compared to the nondiabetic population. Recently, the Bone and Diabetes Working Group of the International Osteoporosis Foundation[123] recommended use of an intervention threshold of a BMD T-score of -2.0 at the hip or spine in patients with diabetes (Figure 4). Although possibly appropriate in Western populations, this proposed adjustment and absolute cut-off may not apply to Asian and the Middle East populations. This working group also suggested a monitoring every 2 years of BMD in diabetes. If significant BMD loss is observed upon two consecutive measurements (≥ 5% in 2 years), or the T-score reaches close to -2.0, treatment should be considered (Figure 4).

Risk assessment tools, such as FRAX, do not entirely capture the elevated risks in patients with T2DM. Therefore, for a given FRAX score, a higher risk of fracture is observed in T2DM patients than in patients without T2DM[5]. As T2DM confers an elevated fracture risk that is not dependent on standard CRFs, it has been suggested that inclusion of T2DM be considered in future FRAX versions[3]. The FRAX calculated fracture risk in diabetes is estimated to be equivalent to an addition of 10 years of age or decreasing the BMD T-score by 0.5 SD[5]. Rheumatoid arthritis input to FRAX as a proxy for the T2DM effect is one option. Clinically, such a FRAX adjustment for T2DM can be useful despite limitations[124].

Osteoporosis therapies-efficacy in diabetes and cautions

Does diabetes modify the effectiveness of medications for osteoporosis? There is very little information available from comparative studies on the efficacy of osteoporosis therapies in diabetes-induced osteoporosis in general and in T1DM specifically. This has been worsened by the fact that diabetes is frequently an exclusion criterion for enrollment in clinical trials. In addition, there are concerns that in the setting of diabetes, antiresorptive therapies that suppress bone turnover may not be as effective[125]. Regarding antiresorptive therapies in people with diabetes, the efficacy of bisphosphonates and raloxifene in diabetic individuals are discussed here. Until now, the efficacy of denosumab in diabetes has been investigated in only one study.

Post hoc analyses of RCTs comparing results in people with diabetes randomized to treatment *vs* placebo have provided the strongest clinical evidence concerning the efficacy of bisphosphonates in diabetic population. In any particular trial, however, the number of diabetic patients is often insufficient to evaluate the fracture-related outcome. Conducted among postmenopausal women in the United States, a post hoc analysis of the Fracture Intervention Trial, showed that the lumbar spine and hip BMDs were increased following alendronate therapy for 3 years relative to placebo in women with T2DM[126]. The size of these effects is comparable in diabetic and nondiabetic women. Risedronate efficacy in diabetic patients is established from the results of three RCTs that were conducted in Japan[127]. Risedronate has similar effects on bone resorption and formation markers and BMD at the lumbar spine in diabetic and nondiabetic patients. Similar antifracture efficacy for bisphosphonates has been reported in diabetic compared with nondiabetic subjects by observational studies [128,129]. No trials or observational studies have assessed whether the efficacy of osteoporosis therapies in the diabetic population differs by BMD T-score. Analyses of two different RCTs of raloxifene, a nonsteroidal selective estrogen receptor modulator, show similar efficacy for diabetic and nondiabetic women for the prevention of vertebral fractures[130,131]. However, raloxifene also has the limitation of lack of efficacy in nonvertebral fractures similar to results in nondiabetic women. A meta-analysis of antiosteoporosis medications in T1DM and T2DM patients indicated that the efficacy of alendronate, risedronate and raloxifene in improving BMD and decreasing fracture rate is comparable between diabetic and nondiabetic individuals [132].

Effects of denosumab in diabetic patients with osteoporosis have been investigated in the subgroup analysis of the FREEDOM study and FREEDOM extension[133]. Long-term denosumab treatment reduced the risk of vertebral fractures and increased BMD in both diabetic and nondiabetic women with osteoporosis. No reduction in nonvertebral fractures has been observed.

Anabolic agents are of special interest in diabetes, which is associated with lower bone formation, in comparison to postmenopausal osteoporosis that is characterized by increased turnover[125]. Rodent studies are available for PTH and sclerostin antibodies. For PTH 1–34 (teriparatide), post hoc analyses of the DANCE observational study show that effects on nonvertebral fracture risk and BMD gain are similar in patients with T2DM and controls. Furthermore, patients with T2DM have a larger increase in femoral neck BMD during 18 mo of treatment with teriparatide in comparison to controls[134]. At present, clinical studies on the effectiveness of anti-sclerostin monoclonal antibody (romosozumab) in patients with diabetes are not available.

Overall, both antiresorptive and anabolic therapies reduce the risk of fractures in diabetic patients. Table 3 summarizes the efficacy of osteoporosis therapies in patients with diabetes.

Special points when diabetic patients receive osteoporosis therapy: For osteoporosis treatment in diabetes, a vitamin D-sufficient status must be attained through supplementation, and current evidence supports the use of both antiresorptive and anabolic agents[135]. People with diabetes may develop some degree of renal impairment and gastrointestinal complications. Therefore, it is imperative to assess renal function and gastrointestinal symptoms prior to starting antiresorptive drugs. Denosumab may be a favored choice in patients with diabetes who are older and/or have a worsening kidney function. Because diabetes is characterized by poor bone quality and low bone turnover, when sequential osteoporosis treatment is considered, an anabolic agent should be administered initially, followed by an antiresorptive drug[135].

A higher frequency of atypical femur fractures and osteonecrosis of the jaw is observed with the use of bisphosphonates and denosumab[125]. There is conflicting evidence on diabetes being associated with an increased incidence of atypical femur fractures[136]. In the oncology population, diabetes is considered a risk factor for the development of osteonecrosis of the jaw. In this population, the combined effects of osteoporosis therapy and diabetes are unknown.

Antiosteoporosis medications and glucose metabolism

The presence of crosstalk between the bone and energy metabolism has been established with animal models. Thus, the possible effects of antiosteoporosis drugs on glucose metabolism should be noted. Specifically, rodent models point out that OC has favorable effects on glucose metabolism[137]. Given that bone resorption inhibitors suppress OC, the concern is there that these therapies might enhance the risk of diabetes. Post hoc analyses of randomized trials of alendronate, zoledronic acid and denosumab indicated that the risk of diabetes is not increased by the use of antiresorptive therapies[138].

Observational studies have also shown that bisphosphonate use is associated with a lower risk of incident diabetes[139,140]. These findings provide reassurance that antiresorptive therapy will not increase the risk of incident diabetes. Mouse studies have found that downregulation of receptor activator of nuclear factor kappa-B signaling leads to improved hepatic insulin sensitivity and plasma glucose levels[141]. This appears to imply that receptor activator of nuclear factor kappa-B ligand blocking may have a favorable effect on diabetes prevention. Clinical trials, however, did not prove any correlation between denosumab (human monoclonal antibody that inhibits receptor activator of nuclear factor kappa-B ligand) treatment and fasting glucose, IR or diabetes risk[138]. Regarding raloxifene effects on glycemic control, a post hoc analysis found no difference in fasting glucose or HbA1c changes over 3 years between raloxifene and placebo in women with and without diabetes[142]. Using the Danish registry data, an observational study reported that raloxifene was associated with a reduced incidence of diabetes[128].

PTH 1–34 improved IR and increased serum OC in T2DM rats[143]. One short study revealed that teriparatide did not affect glucose metabolism after 6 mo of treat-ment [144]. However, another study showed that after 6 mo of treatment with teriparatide (20 μg/d) fasting glucose and Homeostatic Model Assessment for IR index increased in postmenopausal women[145].

Table 3 Effects of osteoporosis medications in patients with type 2 diabetes mellitus

Medication	Effect on glucose metabolism	BMD	Risk of fracture
Alendronate	Reduction in the risk of diabetes	Increase	NA/unchanged
Risedronate	Reduction in the risk of diabetes	Increase	NA
Etidronate	NA	NA	Unchanged
Denosumab	No effect on blood glucoselevels	Increase	Decrease
Raloxifene	Improves insulin sensitivity	NA	Decrease/unchanged
Teriparatide	No effect blood glucose levels	Increase	Unchanged

BMD: Bone mineral density; NA: Not available.

In conclusion, the data indicate that antiosteoporosis medications have minimal, if any, effects on glucose metabolism (Table 3). The findings of a reduction in the risk of developing diabetes with bisphosphonate use merit further investigation. Strategies for the best possible management of patients with T2DM and coexisting osteoporosis have been detailed elsewhere[146]. Figure 5 provides an outline of management.

Osteoporosis targeted pharmacotherapy in T1DM

Given that T1DM is a low bone turnover state, anabolic agents like intermittent recombinant human PTH therapy and antisclerostin agent romosozumab seem to be interesting therapeutic options, but there are no human studies in the T1DM population. Bisphosphonates have shown no difference in efficacy in T1DM compared to T2DM or nondiabetics)[126]. However, caution must be exercised while using bisphosphonates in women of reproductive age. Denosumab increases predominantly cortical BMD, which makes it another intriguing option in T1DM, but there is no data yet. A novel agent, recombinant IGF-1, has shown promising results in T1DM rodent models[147].

Emerging treatment options

A potential new antiosteoporosis treatment, romosozumab, is a monoclonal antibody against sclerostin that causes a loss of osteoblast inhibition along with inhibition of osteoclast activation. Romosozumab improves BMD at different skeletal sites and decreases the risk of fracture compared with placebo or other antiosteoporosis treatments[148]. Because elevated levels of sclerostin in diabetes may contribute to bone disease, it will be interesting to investigate the effect of romosozumab in diabetic patients. Further, research into the role of PTH for bone protection in patients with diabetes can provide interesting insights into its use as it is by far the best treatment for this patient population. Because AGEs play an important role in the pathogenesis of DM and osteoporosis, prevention of AGE-induced glycation of proteins connected with the maintenance of bone health can be a potential way of managing diabetes-induced osteoporosis.

CONCLUSION

Both T1DM and T2DM are associated with bone fragility although *via* different mechanisms. The situation seems more complex in T2DM as BMD is elevated, and the bone quality alterations are multifactorial. The contribution of antidiabetic medications, if any exists, appears limited except through the induction of hypoglycemic episodes responsible for falls. Diabetes-associated osteoporosis and fracture are important complications to consider when evaluating patients with long-standing DM. However, there is no clear consensus on how to screen for fracture risk and when to initiate treatment of osteoporosis in patients with DM. Therefore, realistic measures should be taken, with special attention to the prevention of falls. Good glycemic control is essential for reducing the risk of bone fragility, but hypoglycemia should be avoided and medications with a neutral effect on bone metabolism are preferred. Future research should continue evaluating the structural determinants of bone fragility in diabetes and improving the fracture prediction tools to facilitate timely intervention and fracture prevention. The available data, albeit small, suggest that

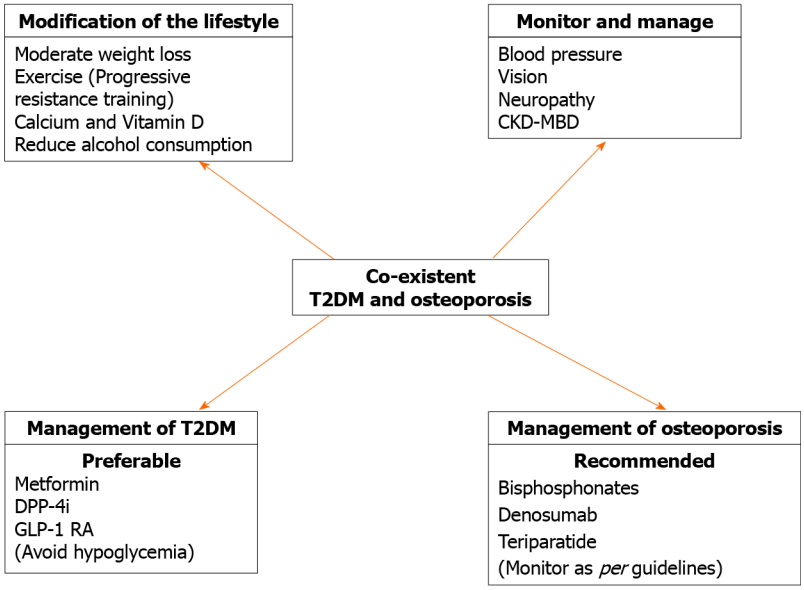

Figure 5 Strategies for treating type 2 diabetes mellitus and concurrent osteoporosis. CKD-MBD: Chronic kidney disease–mineral and bone disorder; DPP-4i: Dipeptidyl-peptidase 4 inhibitor; GLP-1: Glucagon-like peptide-1; T2DM: Type 2 diabetes mellitus.

antiosteoporosis medications are equally effective in patients with and without diabetes. Dedicated trials investigating the effects of new osteoporosis drugs, such as sclerostin antibodies, on bone strength and fracture outcomes in diabetes are needed.

REFERENCES

1 **International Diabetes Federation**. IDF Diabetes Atlas 8th edition 2017. [cited 30 December 2020]. Available from: https://diabetesatlas.org/en/

2 **Napoli N**, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL; IOF Bone and Diabetes Working Group. Mechanisms of diabetes mellitus-induced bone fragility. *Nat Rev Endocrinol* 2017; **13**: 208-219 [PMID: 27658727 DOI: 10.1038/nrendo.2016.153]

3 **Giangregorio LM**, Leslie WD, Lix LM, Johansson H, Oden A, McCloskey E, Kanis JA. FRAX underestimates fracture risk in patients with diabetes. *J Bone Miner Res* 2012; **27**: 301-308 [PMID: 22052532 DOI: 10.1002/jbmr.556]

4 **Hofbauer LC**, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. *J Bone Miner Res* 2007; **22**: 1317-1328 [PMID: 17501667 DOI: 10.1359/jbmr.070510]

5 **Schwartz AV**, Vittinghoff E, Bauer DC, Hillier TA, Strotmeyer ES, Ensrud KE, Donaldson MG, Cauley JA, Harris TB, Koster A, Womack CR, Palermo L, Black DM; Study of Osteoporotic Fractures (SOF) Research Group; Osteoporotic Fractures in Men (MrOS) Research Group; Health, Aging; and Body Composition (Health ABC) Research Group. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *JAMA* 2011; **305**: 2184-2192 [PMID: 21632482 DOI: 10.1001/jama.2011.715]

6 **Fulzele K**, DiGirolamo DJ, Liu Z, Xu J, Messina JL, Clemens TL. Disruption of the insulin-like growth factor type 1 receptor in osteoblasts enhances insulin signaling and action. *J Biol Chem* 2007; **282**: 25649-25658 [PMID: 17553792 DOI: 10.1074/jbc.M700651200]

7 **Cornish J**, Naot D. Amylin and adrenomedullin: novel regulators of bone growth. *Curr Pharm Des* 2002; **8**: 2009-2021 [PMID: 12171515 DOI: 10.2174/1381612023393341]

8 **Terada M**, Inaba M, Yano Y, Hasuma T, Nishizawa Y, Morii H, Otani S. Growth-inhibitory effect of a high glucose concentration on osteoblast-like cells. *Bone* 1998; **22**: 17-23 [PMID: 9437509 DOI: 10.1016/s8756-3282(97)00220-2]

9 **McCarthy AD**, Etcheverry SB, Cortizo AM. Effect of advanced glycation endproducts on the secretion of insulin-like growth factor-I and its binding proteins: role in osteoblast development. *Acta Diabetol* 2001; **38**: 113-122 [PMID: 11827431 DOI: 10.1007/s005920170007]

10 **Picke AK**, Campbell GM, Blüher M, Krügel U, Schmidt FN, Tsourdi E, Winzer M, Rauner M, Vukicevic V, Busse B, Salbach-Hirsch J, Tuckermann JP, Simon JC, Anderegg U, Hofbauer LC, Saalbach A. Thy-1 (CD90) promotes bone formation and protects against obesity. *Sci Transl Med* 2018; **10** [PMID: 30089635 DOI: 10.1126/scitranslmed.aao6806]

11 **Lee SJ**, Lee JY, Sung J. Obesity and Bone Health Revisited: A Mendelian Randomization Study for Koreans. *J Bone Miner Res* 2019; **34**: 1058-1067 [PMID: 30817851 DOI: 10.1002/jbmr.3678]

12 **Robling AG**, Turner CH. Mechanical signaling for bone modeling and remodeling. *Crit Rev*

Eukaryot Gene Expr 2009; **19**: 319-338 [PMID: 19817708 DOI: 10.1615/critreveukargeneexpr.v19.i4.50]

13 **Cao JJ**, Sun L, Gao H. Diet-induced obesity alters bone remodeling leading to decreased femoral trabecular bone mass in mice. *Ann N Y Acad Sci* 2010; **1192**: 292-297 [PMID: 20392249 DOI: 10.1111/j.1749-6632.2009.05252.x]

14 **Janghorbani M**, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol* 2007; **166**: 495-505 [PMID: 17575306 DOI: 10.1093/aje/kwm106]

15 **Shah VN**, Shah CS, Snell-Bergeon JK. Type 1 diabetes and risk of fracture: meta-analysis and review of the literature. *Diabet Med* 2015; **32**: 1134-1142 [PMID: 26096918 DOI: 10.1111/dme.12734]

16 **Vestergaard P**. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes--a meta-analysis. *Osteoporos Int* 2007; **18**: 427-444 [PMID: 17068657 DOI: 10.1007/s00198-006-0253-4]

17 **Weber DR**, Haynes K, Leonard MB, Willi SM, Denburg MR. Type 1 diabetes is associated with an increased risk of fracture across the life span: a population-based cohort study using The Health Improvement Network (THIN). *Diabetes Care* 2015; **38**: 1913-1920 [PMID: 26216874 DOI: 10.2337/dc15-0783]

18 **Miao J**, Brismar K, Nyrén O, Ugarph-Morawski A, Ye W. Elevated hip fracture risk in type 1 diabetic patients: a population-based cohort study in Sweden. *Diabetes Care* 2005; **28**: 2850-2855 [PMID: 16306544 DOI: 10.2337/diacare.28.12.2850]

19 **Vestergaard P**, Rejnmark L, Mosekilde L. Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. *Diabetologia* 2005; **48**: 1292-1299 [PMID: 15909154 DOI: 10.1007/s00125-005-1786-3]

20 **Hough FS**, Pierroz DD, Cooper C, Ferrari SL; IOF CSA Bone and Diabetes Working Group. MECHANISMS IN ENDOCRINOLOGY: Mechanisms and evaluation of bone fragility in type 1 diabetes mellitus. *Eur J Endocrinol* 2016; **174**: R127-R138 [PMID: 26537861 DOI: 10.1530/EJE-15-0820]

21 **Hamed EA**, Faddan NH, Elhafeez HA, Sayed D. Parathormone--25(OH)-vitamin D axis and bone status in children and adolescents with type 1 diabetes mellitus. *Pediatr Diabetes* 2011; **12**: 536-546 [PMID: 21426456 DOI: 10.1111/j.1399-5448.2010.00739.x]

22 **Zhu Q**, Xu J, Zhou M, Lian X, Shi J. Association between type 1 diabetes mellitus and reduced bone mineral density in children: a meta-analysis. *Osteoporos Int* 2021 [PMID: 33404757 DOI: 10.1007/s00198-020-05715-3]

23 **Fuusager GB**, Christesen HT, Milandt N, Schou AJ. Glycemic control and bone mineral density in children and adolescents with type 1 diabetes. *Pediatr Diabetes* 2019; **20**: 629-636 [PMID: 31017353 DOI: 10.1111/pedi.12861]

24 **Gunczler P**, Lanes R, Paz-Martinez V, Martins R, Esaa S, Colmenares V, Weisinger JR. Decreased lumbar spine bone mass and low bone turnover in children and adolescents with insulin dependent diabetes mellitus followed longitudinally. *J Pediatr Endocrinol Metab* 1998; **11**: 413-419 [PMID: 11517957 DOI: 10.1515/jpem.1998.11.3.413]

25 **Eller-Vainicher C**, Zhukouskaya VV, Tolkachev YV, Koritko SS, Cairoli E, Grossi E, Beck-Peccoz P, Chiodini I, Shepelkevich AP. Low bone mineral density and its predictors in type 1 diabetic patients evaluated by the classic statistics and artificial neural network analysis. *Diabetes Care* 2011; **34**: 2186-2191 [PMID: 21852680 DOI: 10.2337/dc11-0764]

26 **Clausen P**, Feldt-Rasmussen B, Jacobsen P, Rossing K, Parving HH, Nielsen PK, Feldt-Rasmussen U, Olgaard K. Microalbuminuria as an early indicator of osteopenia in male insulin-dependent diabetic patients. *Diabet Med* 1997; **14**: 1038-1043 [PMID: 9455931 DOI: 10.1002/(SICI)1096-9136(199712)14:12<1038::AID-DIA509>3.0.CO;2-1]

27 **Shanbhogue VV**, Hansen S, Frost M, Jørgensen NR, Hermann AP, Henriksen JE, Brixen K. Bone Geometry, Volumetric Density, Microarchitecture, and Estimated Bone Strength Assessed by HR-pQCT in Adult Patients With Type 1 Diabetes Mellitus. *J Bone Miner Res* 2015; **30**: 2188-2199 [PMID: 26096924 DOI: 10.1002/jbmr.2573]

28 **Kemink SA**, Hermus AR, Swinkels LM, Lutterman JA, Smals AG. Osteopenia in insulin-dependent diabetes mellitus; prevalence and aspects of pathophysiology. *J Endocrinol Invest* 2000; **23**: 295-303 [PMID: 10882147 DOI: 10.1007/BF03343726]

29 **Pater A**, Sypniewska G, Pilecki O. Biochemical markers of bone cell activity in children with type 1 diabetes mellitus. *J Pediatr Endocrinol Metab* 2010; **23**: 81-86 [PMID: 20432810 DOI: 10.1515/jpem.2010.23.1-2.81]

30 **Armas LA**, Akhter MP, Drincic A, Recker RR. Trabecular bone histomorphometry in humans with Type 1 Diabetes Mellitus. *Bone* 2012; **50**: 91-96 [PMID: 22001578 DOI: 10.1016/j.bone.2011.09.055]

31 **Verroken C**, Pieters W, Beddeleem L, Goemaere S, Zmierczak HG, Shadid S, Kaufman JM, Lapauw B. Cortical Bone Size Deficit in Adult Patients With Type 1 Diabetes Mellitus. *J Clin Endocrinol Metab* 2017; **102**: 2887-2895 [PMID: 28531321 DOI: 10.1210/jc.2017-00620]

32 **Abdalrahaman N**, McComb C, Foster JE, McLean J, Lindsay RS, McClure J, McMillan M, Drummond R, Gordon D, McKay GA, Shaikh MG, Perry CG, Ahmed SF. Deficits in Trabecular Bone Microarchitecture in Young Women With Type 1 Diabetes Mellitus. *J Bone Miner Res* 2015; **30**: 1386-1393 [PMID: 25627460 DOI: 10.1002/jbmr.2465]

33 **Devaraja J**, Jacques R, Paggiosi M, Clark C, Dimitri P. Impact of Type 1 Diabetes Mellitus on Skeletal Integrity and Strength in Adolescents as Assessed by HRpQCT. *JBMR Plus* 2020; **4**: e10422 [PMID: 33210068 DOI: 10.1002/jbm4.10422]

34 **Shah VN**, Carpenter RD, Ferguson VL, Schwartz AV. Bone health in type 1 diabetes. *CurrOpinEndocrinol Diabetes Obes* 2018; **25**: 231-236 [PMID: 29794498 DOI: 10.1097/MED.0000000000000421]

35 **Khan TS**, Fraser LA. Type 1 diabetes and osteoporosis: from molecular pathways to bone phenotype. *J Osteoporos* 2015; **2015**: 174186 [PMID: 25874154 DOI: 10.1155/2015/174186]

36 **Wang H**, Ba Y, Xing Q, Du JL. Diabetes mellitus and the risk of fractures at specific sites: a meta-analysis. *BMJ Open* 2019; **9**: e024067 [PMID: 30610024 DOI: 10.1136/bmjopen-2018-024067]

37 **Moayeri A**, Mohamadpour M, Mousavi SF, Shirzadpour E, Mohamadpour S, Amraei M. Fracture risk in patients with type 2 diabetes mellitus and possible risk factors: a systematic review and meta-analysis. *Ther Clin Risk Manag* 2017; **13**: 455-468 [PMID: 28442913 DOI: 10.2147/TCRM.S131945]

38 **Jia P**, Bao L, Chen H, Yuan J, Liu W, Feng F, Li J, Tang H. Risk of low-energy fracture in type 2 diabetes patients: a meta-analysis of observational studies. *Osteoporos Int* 2017; **28**: 3113-3121 [PMID: 28795239 DOI: 10.1007/s00198-017-4183-0]

39 **Vilaca T**, Schini M, Harnan S, Sutton A, Poku E, Allen IE, Cummings SR, Eastell R. The risk of hip and non-vertebral fractures in type 1 and type 2 diabetes: A systematic review and meta-analysis update. *Bone* 2020; **137**: 115457 [PMID: 32480023 DOI: 10.1016/j.bone.2020.115457]

40 **Dytfeld J**, Michalak M. Type 2 diabetes and risk of low-energy fractures in postmenopausal women: meta-analysis of observational studies. *Aging Clin Exp Res* 2017; **29**: 301-309 [PMID: 27072353 DOI: 10.1007/s40520-016-0562-1]

41 **Fan Y**, Wei F, Lang Y, Liu Y. Diabetes mellitus and risk of hip fractures: a meta-analysis. *Osteoporos Int* 2016; **27**: 219-228 [PMID: 26264604 DOI: 10.1007/s00198-015-3279-7]

42 **Ni Y**, Fan D. Diabetes mellitus is a risk factor for low bone mass-related fractures: A meta-analysis of cohort studies. *Medicine (Baltimore)* 2017; **96**: e8811 [PMID: 29390417 DOI: 10.1097/MD.0000000000008811]

43 **Koromani F**, Oei L, Shevroja E, Trajanoska K, Schoufour J, Muka T, Franco OH, Ikram MA, Zillikens MC, Uitterlinden AG, Krestin GP, Anastassiades T, Josse R, Kaiser SM, Goltzman D, Lentle BC, Prior JC, Leslie WD, McCloskey E, Lamy O, Hans D, Oei EH, Rivadeneira F. Vertebral Fractures in Individuals With Type 2 Diabetes: More Than Skeletal Complications Alone. *Diabetes Care* 2020; **43**: 137-144 [PMID: 31658976 DOI: 10.2337/dc19-0925]

44 **Liu J**, Cao L, Qian YW, Chen ZX, Guo SF, Sun WQ, He ZR. The association between risk of limb fracture and type 2 diabetes mellitus. *Oncotarget* 2018; **9**: 31302-31310 [PMID: 30131856 DOI: 10.18632/oncotarget.23937]

45 **Vilaca T**, Walsh J, Eastell R. Discordant pattern of peripheral fractures in diabetes: a meta-analysis on the risk of wrist and ankle fractures. *Osteoporos Int* 2019; **30**: 135-143 [PMID: 30306223 DOI: 10.1007/s00198-018-4717-0]

46 **Ma L**, Oei L, Jiang L, Estrada K, Chen H, Wang Z, Yu Q, Zillikens MC, Gao X, Rivadeneira F. Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis of observational studies. *Eur J Epidemiol* 2012; **27**: 319-332 [PMID: 22451239 DOI: 10.1007/s10654-012-9674-x]

47 **Jang M**, Kim H, Lea S, Oh S, Kim JS, Oh B. Effect of duration of diabetes on bone mineral density: a population study on East Asian males. *BMC EndocrDisord* 2018; **18**: 61 [PMID: 30185190 DOI: 10.1186/s12902-018-0290-y]

48 **Lee HS**, Yoon JS, Park KJ, Lim JS, Hwang JS. The Relationship Between Bone Mineral Density and Type 2 Diabetes in Obese Children and Adolescents at the Time of Initial Diagnosis. *HormMetab Res* 2019; **51**: 42-46 [PMID: 30340234 DOI: 10.1055/a-0755-2799]

49 **Asokan AG**, Jaganathan J, Philip R, Soman RR, Sebastian ST, Pullishery F. Evaluation of bone mineral density among type 2 diabetes mellitus patients in South Karnataka. *J Nat Sci Biol Med* 2017; **8**: 94-98 [PMID: 28250682 DOI: 10.4103/0976-9668.198363]

50 **Ho-Pham LT**, Nguyen TV. Association between trabecular bone score and type 2 diabetes: a quantitative update of evidence. *Osteoporos Int* 2019; **30**: 2079-2085 [PMID: 31214749 DOI: 10.1007/s00198-019-05053-z]

51 **Choi YJ**, Ock SY, Jin Y, Lee JS, Kim SH, Chung Y-. Urinary Pentosidine levels negatively associates with trabecular bone scores in patients with type 2 diabetes mellitus. *Osteoporos Int* 2018; **29**: 907-915 [PMID: 29322222 DOI: 10.1007/s00198-017-4359-7]

52 **Burghardt AJ**, Issever AS, Schwartz AV, Davis KA, Masharani U, Majumdar S, Link TM. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2010; **95**: 5045-5055 [PMID: 20719835 DOI: 10.1210/jc.2010-0226]

53 **Patsch JM**, Burghardt AJ, Yap SP, Baum T, Schwartz AV, Joseph GB, Link TM. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. *J Bone Miner Res* 2013; **28**: 313-324 [PMID: 22991256 DOI: 10.1002/jbmr.1763]

54 **Shanbhogue VV**, Hansen S, Frost M, Jørgensen NR, Hermann AP, Henriksen JE, Brixen K. Compromised cortical bone compartment in type 2 diabetes mellitus patients with microvascular disease. *Eur J Endocrinol* 2016; **174**: 115-124 [PMID: 26537860 DOI: 10.1530/EJE-15-0860]

55 **Heilmeier U**, Cheng K, Pasco C, Parrish R, Nirody J, Patsch JM, Zhang CA, Joseph GB, Burghardt

AJ, Schwartz AV, Link TM, Kazakia G. Cortical bone laminar analysis reveals increased midcortical and periosteal porosity in type 2 diabetic postmenopausal women with history of fragility fractures compared to fracture-free diabetics. *Osteoporos Int* 2016; **27**: 2791-2802 [PMID: 27154435 DOI: 10.1007/s00198-016-3614-7]

56 **Samakkarnthai P**, Sfeir JG, Atkinson EJ, Achenbach SJ, Wennberg PW, Dyck PJ, Tweed AJ, Volkman TL, Amin S, Farr JN, Vella A, Drake MT, Khosla S. Determinants of Bone Material Strength and Cortical Porosity in Patients with Type 2 Diabetes Mellitus. *J Clin Endocrinol Metab* 2020; **105** [PMID: 32556277 DOI: 10.1210/clinem/dgaa388]

57 **de Waard EAC**, de Jong JJA, Koster A, Savelberg HHCM, van Geel TA, Houben AJHM, Schram MT, Dagnelie PC, van der Kallen CJ, Sep SJS, Stehouwer CDA, Schaper NC, Berendschot TTJM, Schouten JSAG, Geusens PPMM, van den Bergh JPW. The association between diabetes status, HbA1c, diabetes duration, microvascular disease, and bone quality of the distal radius and tibia as measured with high-resolution peripheral quantitative computed tomography-The Maastricht Study. *Osteoporos Int* 2018; **29**: 2725-2738 [PMID: 30209523 DOI: 10.1007/s00198-018-4678-3]

58 **Farr JN**, Drake MT, Amin S, Melton LJ 3rd, McCready LK, Khosla S. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. *J Bone Miner Res* 2014; **29**: 787-795 [PMID: 24123088 DOI: 10.1002/jbmr.2106]

59 **Nilsson AG**, Sundh D, Johansson L, Nilsson M, Mellström D, Rudäng R, Zoulakis M, Wallander M, Darelid A, Lorentzon M. Type 2 Diabetes Mellitus Is Associated With Better Bone Microarchitecture But Lower Bone Material Strength and Poorer Physical Function in Elderly Women: A Population-Based Study. *J Bone Miner Res* 2017; **32**: 1062-1071 [PMID: 27943408 DOI: 10.1002/jbmr.3057]

60 **Sundh D**, Rudäng R, Zoulakis M, Nilsson AG, Darelid A, Lorentzon M. A High Amount of Local Adipose Tissue Is Associated With High Cortical Porosity and Low Bone Material Strength in Older Women. *J Bone Miner Res* 2016; **31**: 749-757 [PMID: 26588353 DOI: 10.1002/jbmr.2747]

61 **Starup-Linde J**, Eriksen SA, Lykkeboe S, Handberg A, Vestergaard P. Biochemical markers of bone turnover in diabetes patients--a meta-analysis, and a methodological study on the effects of glucose on bone markers. *Osteoporos Int* 2014; **25**: 1697-1708 [PMID: 24676844 DOI: 10.1007/s00198-014-2676-7]

62 **Hygum K**, Starup-Linde J, Harsløf T, Vestergaard P, Langdahl BL. MECHANISMS IN ENDOCRINOLOGY: Diabetes mellitus, a state of low bone turnover - a systematic review and meta-analysis. *Eur J Endocrinol* 2017; **176**: R137-R157 [PMID: 28049653 DOI: 10.1530/EJE-16-0652]

63 **Manolagas SC**, Almeida M. Gone with the Wnts: beta-catenin, T-cell factor, forkhead box O, and oxidative stress in age-dependent diseases of bone, lipid, and glucose metabolism. *Mol Endocrinol* 2007; **21**: 2605-2614 [PMID: 17622581 DOI: 10.1210/me.2007-0259]

64 **Heilmeier U**, Carpenter DR, Patsch JM, Harnish R, Joseph GB, Burghardt AJ, Baum T, Schwartz AV, Lang TF, Link TM. Volumetric femoral BMD, bone geometry, and serum sclerostin levels differ between type 2 diabetic postmenopausal women with and without fragility fractures. *Osteoporos Int* 2015; **26**: 1283-1293 [PMID: 25582311 DOI: 10.1007/s00198-014-2988-7]

65 **Krakauer JC**, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM. Bone loss and bone turnover in diabetes. *Diabetes* 1995; **44**: 775-782 [PMID: 7789645 DOI: 10.2337/diab.44.7.775]

66 **Manavalan JS**, Cremers S, Dempster DW, Zhou H, Dworakowski E, Kode A, Kousteni S, Rubin MR. Circulating osteogenic precursor cells in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2012; **97**: 3240-3250 [PMID: 22740707 DOI: 10.1210/jc.2012-1546]

67 **Shanbhogue VV**, Mitchell DM, Rosen CJ, Bouxsein ML. Type 2 diabetes and the skeleton: new insights into sweet bones. *Lancet Diabetes Endocrinol* 2016; **4**: 159-173 [PMID: 26365605 DOI: 10.1016/S2213-8587(15)00283-1]

68 **de Waard EAC**, Driessen JHM, de Jong JJA, van Geel TACM, Henry RMA, van Onzenoort HAW, Schram MT, Dagnelie PC, van der Kallen CJ, Sep SJS, Stehouwer CDA, Schaper NC, Koster A, Savelberg HHCM, Neef C, Geusens PPMM, de Vries F, van den Bergh JPW. The association between insulin use and volumetric bone mineral density, bone micro-architecture and bone strength of the distal radius in patients with type 2 diabetes - The Maastricht study. *Bone* 2017; **101**: 156-161 [PMID: 28487133 DOI: 10.1016/j.bone.2017.05.004]

69 **Wallander M**, Axelsson KF, Nilsson AG, Lundh D, Lorentzon M. Type 2 Diabetes and Risk of Hip Fractures and Non-Skeletal Fall Injuries in the Elderly: A Study From the Fractures and Fall Injuries in the Elderly Cohort (FRAILCO). *J Bone Miner Res* 2017; **32**: 449-460 [PMID: 27664946 DOI: 10.1002/jbmr.3002]

70 **Losada-Grande E**, Hawley S, Soldevila B, Martinez-Laguna D, Nogues X, Diez-Perez A, Puig-Domingo M, Mauricio D, Prieto-Alhambra D. Insulin use and Excess Fracture Risk in Patients with Type 2 Diabetes: A Propensity-Matched cohort analysis. *Sci Rep* 2017; **7**: 3781 [PMID: 28630427 DOI: 10.1038/s41598-017-03748-z]

71 **Pscherer S**, Kostev K, Dippel FW, Rathmann W. Fracture risk in patients with type 2 diabetes under different antidiabetic treatment regimens: a retrospective database analysis in primary care. *Diabetes MetabSyndrObes* 2016; **9**: 17-23 [PMID: 26929655 DOI: 10.2147/DMSO.S101370]

72 **Tolosa MJ**, Chuguransky SR, Sedlinsky C, Schurman L, McCarthy AD, Molinuevo MS, Cortizo AM. Insulin-deficient diabetes-induced bone microarchitecture alterations are associated with a decrease in the osteogenic potential of bone marrow progenitor cells: preventive effects of

metformin. *Diabetes Res Clin Pract* 2013; **101**: 177-186 [PMID: 23806481 DOI: 10.1016/j.diabres.2013.05.016]

73 **Mai QG**, Zhang ZM, Xu S, Lu M, Zhou RP, Zhao L, Jia CH, Wen ZH, Jin DD, Bai XC. Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats. *J Cell Biochem* 2011; **112**: 2902-2909 [PMID: 21618594 DOI: 10.1002/jcb.23206]

74 **Hidayat K**, Du X, Wu MJ, Shi BM. The use of metformin, insulin, sulphonylureas, and thiazolidinediones and the risk of fracture: Systematic review and meta-analysis of observational studies. *Obes Rev* 2019; **20**: 1494-1503 [PMID: 31250977 DOI: 10.1111/obr.12885]

75 **Zhang Z**, Cao Y, Tao Y, E M, Tang J, Liu Y, Li F. Sulfonylurea and fracture risk in patients with type 2 diabetes mellitus: A meta-analysis. *Diabetes Res Clin Pract* 2020; **159**: 107990 [PMID: 31866530 DOI: 10.1016/j.diabres.2019.107990]

76 **Choi HJ**, Park C, Lee YK, Ha YC, Jang S, Shin CS. Risk of fractures and diabetes medications: a nationwide cohort study. *Osteoporos Int* 2016; **27**: 2709-2715 [PMID: 27080708 DOI: 10.1007/s00198-016-3595-6]

77 **Ali AA**, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL. Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. *Endocrinology* 2005; **146**: 1226-1235 [PMID: 15591153 DOI: 10.1210/en.2004-0735]

78 **Zinman B**, Haffner SM, Herman WH, Holman RR, Lachin JM, Kravitz BG, Paul G, Jones NP, Aftring RP, Viberti G, Kahn SE; ADOPT Study Group. Effect of rosiglitazone, metformin, and glyburide on bone biomarkers in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2010; **95**: 134-142 [PMID: 19875477 DOI: 10.1210/jc.2009-0572]

79 **Zhu ZN**, Jiang YF, Ding T. Risk of fracture with thiazolidinediones: an updated meta-analysis of randomized clinical trials. *Bone* 2014; **68**: 115-123 [PMID: 25173606 DOI: 10.1016/j.bone.2014.08.010]

80 **Wang C**, Xiao F, Qu X, Zhai Z, Hu G, Chen X, Zhang X. Sitagliptin, An Anti-diabetic Drug, Suppresses Estrogen Deficiency-Induced Osteoporosis*In Vivo* and Inhibits RANKL-Induced Osteoclast Formation and Bone Resorption *In Vitro*. *Front Pharmacol* 2017; **8**: 407 [PMID: 28713268 DOI: 10.3389/fphar.2017.00407]

81 **Fu J**, Zhu J, Hao Y, Guo C, Zhou Z. Dipeptidyl peptidase-4 inhibitors and fracture risk: an updated meta-analysis of randomized clinical trials. *Sci Rep* 2016; **6**: 29104 [PMID: 27384445 DOI: 10.1038/srep29104]

82 **Nuche-Berenguer B**, Moreno P, Esbrit P, Dapía S, Caeiro JR, Cancelas J, Haro-Mora JJ, Villanueva-Peñacarrillo ML. Effect of GLP-1 treatment on bone turnover in normal, type 2 diabetic, and insulin-resistant states. *Calcif Tissue Int* 2009; **84**: 453-461 [PMID: 19219381 DOI: 10.1007/s00223-009-9220-3]

83 **Bunck MC**, Eliasson B, Cornér A, Heine RJ, Shaginian RM, Taskinen MR, Yki-Järvinen H, Smith U, Diamant M. Exenatide treatment did not affect bone mineral density despite body weight reduction in patients with type 2 diabetes. *Diabetes ObesMetab* 2011; **13**: 374-377 [PMID: 21205127 DOI: 10.1111/j.1463-1326.2010.01355.x]

84 **Zhang YS**, Weng WY, Xie BC, Meng Y, Hao YH, Liang YM, Zhou ZK. Glucagon-like peptide-1 receptor agonists and fracture risk: a network meta-analysis of randomized clinical trials. *Osteoporos Int* 2018; **29**: 2639-2644 [PMID: 30083774 DOI: 10.1007/s00198-018-4649-8]

85 **Bilezikian JP**, Watts NB, Usiskin K, Polidori D, Fung A, Sullivan D, Rosenthal N. Evaluation of Bone Mineral Density and Bone Biomarkers in Patients With Type 2 Diabetes Treated With Canagliflozin. *J Clin Endocrinol Metab* 2016; **101**: 44-51 [PMID: 26580234 DOI: 10.1210/jc.2015-1860]

86 **Cheng L**, Li YY, Hu W, Bai F, Hao HR, Yu WN, Mao XM. Risk of bone fracture associated with sodium-glucose cotransporter-2 inhibitor treatment: A meta-analysis of randomized controlled trials. *Diabetes Metab* 2019; **45**: 436-445 [PMID: 30738154 DOI: 10.1016/j.diabet.2019.01.010]

87 **Yu EW**, Bouxsein ML, Putman MS, Monis EL, Roy AE, Pratt JS, Butsch WS, Finkelstein JS. Two-year changes in bone density after Roux-en-Y gastric bypass surgery. *J Clin Endocrinol Metab* 2015; **100**: 1452-1459 [PMID: 25646793 DOI: 10.1210/jc.2014-4341]

88 **Fraser LA**, Pritchard J, Ioannidis G, Giangegorio LM, Adachi JD, Papaioannou A, Leslie WD. Clinical risk factors for fracture in diabetes: a matched cohort analysis. *J Clin Densitom* 2011; **14**: 416-421 [PMID: 21880525 DOI: 10.1016/j.jocd.2011.06.007]

89 **Majumdar SR**, Leslie WD, Lix LM, Morin SN, Johansson H, Oden A, McCloskey EV, Kanis JA. Longer Duration of Diabetes Strongly Impacts Fracture Risk Assessment: The Manitoba BMD Cohort. *J Clin Endocrinol Metab* 2016; **101**: 4489-4496 [PMID: 27603908 DOI: 10.1210/jc.2016-2569]

90 **Lee RH**, Sloane R, Pieper C, Lyles KW, Adler RA, Van Houtven C, LaFleur J, Colón-Emeric C. Clinical Fractures Among Older Men With Diabetes Are Mediated by Diabetic Complications. *J Clin Endocrinol Metab* 2018; **103**: 281-287 [PMID: 29099931 DOI: 10.1210/jc.2017-01593]

91 **Strotmeyer ES**, Cauley JA, Schwartz AV, Nevitt MC, Resnick HE, Bauer DC, Tylavsky FA, de Rekeneire N, Harris TB, Newman AB. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: the health, aging, and body composition study. *Arch Intern Med* 2005; **165**: 1612-1617 [PMID: 16043679 DOI: 10.1001/archinte.165.14.1612]

92 **Poiana C**, Capatina C. Fracture Risk Assessment in Patients With Diabetes Mellitus. *J Clin Densitom* 2017; **20**: 432-443 [PMID: 28716499 DOI: 10.1016/j.jocd.2017.06.011]

93 **World Health Organization**. 2008 Assessment of osteoporosis at the primary health-care level. Technical Report. WHO Collaborating Centre, University of Sheffield, UK. [cited 30 December 2020]. Available from: http://www.shef.ac.uk/FRAX/index.htm

94 **Schacter GI**, Leslie WD. DXA-Based Measurements in Diabetes: Can They Predict Fracture Risk? *Calcif Tissue Int* 2017; **100**: 150-164 [PMID: 27591864 DOI: 10.1007/s00223-016-0191-x]

95 **Yu EW**, Putman MS, Derrico N, Abrishamanian-Garcia G, Finkelstein JS, Bouxsein ML. Defects in cortical microarchitecture among African-American women with type 2 diabetes. *Osteoporos Int* 2015; **26**: 673-679 [PMID: 25398431 DOI: 10.1007/s00198-014-2927-7]

96 **Samelson EJ**, Demissie S, Cupples LA, Zhang X, Xu H, Liu CT, Boyd SK, McLean RR, Broe KE, Kiel DP, Bouxsein ML. Diabetes and Deficits in Cortical Bone Density, Microarchitecture, and Bone Size: Framingham HR-pQCT Study. *J Bone Miner Res* 2018; **33**: 54-62 [PMID: 28929525 DOI: 10.1002/jbmr.3240]

97 **Furst JR**, Bandeira LC, Fan WW, Agarwal S, Nishiyama KK, McMahon DJ, Dworakowski E, Jiang H, Silverberg SJ, Rubin MR. Advanced Glycation Endproducts and Bone Material Strength in Type 2 Diabetes. *J Clin Endocrinol Metab* 2016; **101**: 2502-2510 [PMID: 27115060 DOI: 10.1210/jc.2016-1437]

98 **Leslie WD**, Aubry-Rozier B, Lamy O, Hans D; Manitoba Bone Density Program. TBS (trabecular bone score) and diabetes-related fracture risk. *J Clin Endocrinol Metab* 2013; **98**: 602-609 [PMID: 23341489 DOI: 10.1210/jc.2012-3118]

99 **Kim JH**, Choi HJ, Ku EJ, Kim KM, Kim SW, Cho NH, Shin CS. Trabecular bone score as an indicator for skeletal deterioration in diabetes. *J Clin Endocrinol Metab* 2015; **100**: 475-482 [PMID: 25368976 DOI: 10.1210/jc.2014-2047]

100 **Martineau P**, Leslie WD, Johansson H, Oden A, McCloskey EV, Hans D, Kanis JA. Clinical Utility of Using Lumbar Spine Trabecular Bone Score to Adjust Fracture Probability: The Manitoba BMD Cohort. *J Bone Miner Res* 2017; **32**: 1568-1574 [PMID: 28276598 DOI: 10.1002/jbmr.3124]

101 **Neumann T**, Lodes S, Kästner B, Lehmann T, Hans D, Lamy O, Müller UA, Wolf G, Sämann A. Trabecular bone score in type 1 diabetes--a cross-sectional study. *Osteoporos Int* 2016; **27**: 127-133 [PMID: 26187124 DOI: 10.1007/s00198-015-3222-y]

102 **Jiang N**, Xia W. Assessment of bone quality in patients with diabetes mellitus. *OsteoporosInt* 2018; **29**: 1721-1736 [PMID: 29736760 DOI: 10.1007/s00198-018-4532-7]

103 **Andrade VFC**, Chula DC, Sabbag FP, Cavalheiro DDDS, Bavia L, Ambrósio AR, da Costa CRV, Dos Reis LM, Borba VZC, Moreira CA. Bone Histomorphometry in Young Patients With Type 2 Diabetes is Affected by Disease Control and Chronic Complications. *J Clin Endocrinol Metab* 2020; **105** [PMID: 31587051 DOI: 10.1210/clinem/dgz070]

104 **Madsen JOB**, Jørgensen NR, Pociot F, Johannesen J. Bone turnover markers in children and adolescents with type 1 diabetes-A systematic review. *Pediatr Diabetes* 2019; **20**: 510-522 [PMID: 30941847 DOI: 10.1111/pedi.12853]

105 **Sarkar PD**, Choudhury AB. Relationships between serum osteocalcin levels versus blood glucose, insulin resistance and markers of systemic inflammation in central Indian type 2 diabetic patients. *Eur Rev Med Pharmacol Sci* 2013; **17**: 1631-1635 [PMID: 23832730]

106 **Yamamoto M**, Yamaguchi T, Nawata K, Yamauchi M, Sugimoto T. Decreased PTH levels accompanied by low bone formation are associated with vertebral fractures in postmenopausal women with type 2 diabetes. *J Clin Endocrinol Metab* 2012; **97**: 1277-1284 [PMID: 22337915 DOI: 10.1210/jc.2011-2537]

107 **Reyes-García R**, Rozas-Moreno P, López-Gallardo G, García-Martín A, Varsavsky M, Avilés-Perez MD, Muñoz-Torres M. Serum levels of bone resorption markers are decreased in patients with type 2 diabetes. *Acta Diabetol* 2013; **50**: 47-52 [PMID: 22042129 DOI: 10.1007/s00592-011-0347-0]

108 **Jiajue R**, Jiang Y, Wang O, Li M, Xing X, Cui L, Yin J, Xu L, Xia W. Suppressed bone turnover was associated with increased osteoporotic fracture risks in non-obese postmenopausal Chinese women with type 2 diabetes mellitus. *Osteoporos Int* 2014; **25**: 1999-2005 [PMID: 24760246 DOI: 10.1007/s00198-014-2714-5]

109 **Ardawi MS**, Akhbar DH, Alshaikh A, Ahmed MM, Qari MH, Rouzi AA, Ali AY, Abdulrafee AA, Saeda MY. Increased serum sclerostin and decreased serum IGF-1 are associated with vertebral fractures among postmenopausal women with type-2 diabetes. *Bone* 2013; **56**: 355-362 [PMID: 23845326 DOI: 10.1016/j.bone.2013.06.029]

110 **Starup-Linde J**, Vestergaard P. Biochemical bone turnover markers in diabetes mellitus - A systematic review. *Bone* 2016; **82**: 69-78 [PMID: 25722065 DOI: 10.1016/j.bone.2015.02.019]

111 **Gordon CM**, Leonard MB, Zemel BS; International Society for Clinical Densitometry. 2013 Pediatric Position Development Conference: executive summary and reflections. *J Clin Densitom* 2014; **17**: 219-224 [PMID: 24657108 DOI: 10.1016/j.jocd.2014.01.007]

112 **Slavcheva-Prodanova O**, Konstantinova M, Tsakova A, Savova R, Archinkova M. Bone Health Index and bone turnover in pediatric patients with type 1 diabetes mellitus and poor metabolic control. *Pediatr Diabetes* 2020; **21**: 88-97 [PMID: 31599085 DOI: 10.1111/pedi.12930]

113 **Zhukouskaya VV**, Eller-Vainicher C, Shepelkevich AP, Dydyshko Y, Cairoli E, Chiodini I. Bone health in type 1 diabetes: focus on evaluation and treatment in clinical practice. *J Endocrinol Invest* 2015; **38**: 941-950 [PMID: 25863666 DOI: 10.1007/s40618-015-0284-9]

114 **Picke AK**, Sylow L, Møller LLV, Kjøbsted R, Schmidt FN, Steejn MW, Salbach-Hirsch J, Hofbauer C, Blüher M, Saalbach A, Busse B, Rauner M, Hofbauer LC. Differential effects of high-fat diet and exercise training on bone and energy metabolism. *Bone* 2018; **116**: 120-134 [PMID: 30036679 DOI:

10.1016/j.bone.2018.07.015]
115 **Scott D**, Seibel M, Cumming R, Naganathan V, Blyth F, Le Couteur DG, Handelsman DJ, Waite LM, Hirani V. Sarcopenic Obesity and Its Temporal Associations With Changes in Bone Mineral Density, Incident Falls, and Fractures in Older Men: The Concord Health and Ageing in Men Project. *J Bone Miner Res* 2017; **32**: 575-583 [PMID: 27736026 DOI: 10.1002/jbmr.3016]
116 **Schwartz AV**, Margolis KL, Sellmeyer DE, Vittinghoff E, Ambrosius WT, Bonds DE, Josse RG, Schnall AM, Simmons DL, Hue TF, Palermo L, Hamilton BP, Green JB, Atkinson HH, O'Connor PJ, Force RW, Bauer DC. Intensive glycemic control is not associated with fractures or falls in the ACCORD randomized trial. *Diabetes Care* 2012; **35**: 1525-1531 [PMID: 22723583 DOI: 10.2337/dc11-2184]
117 **Vavanikunnel J**, Charlier S, Becker C, Schneider C, Jick SS, Meier CR, Meier C. Association Between Glycemic Control and Risk of Fracture in Diabetic Patients: A Nested Case-Control Study. *J Clin Endocrinol Metab* 2019; **104**: 1645-1654 [PMID: 30657918 DOI: 10.1210/jc.2018-01879]
118 **Chen SC**, Shepherd S, McMillan M, McNeilly J, Foster J, Wong SC, Robertson KJ, Ahmed SF. Skeletal Fragility and Its Clinical Determinants in Children With Type 1 Diabetes. *J Clin Endocrinol Metab* 2019; **104**: 3585-3594 [PMID: 30848792 DOI: 10.1210/jc.2019-00084]
119 **Oei L**, Zillikens MC, Dehghan A, Buitendijk GH, Castaño-Betancourt MC, Estrada K, Stolk L, Oei EH, van Meurs JB, Janssen JA, Hofman A, van Leeuwen JP, Witteman JC, Pols HA, Uitterlinden AG, Klaver CC, Franco OH, Rivadeneira F. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam Study. *Diabetes Care* 2013; **36**: 1619-1628 [PMID: 23315602 DOI: 10.2337/dc12-1188]
120 **Li CI**, Liu CS, Lin WY, Meng NH, Chen CC, Yang SY, Chen HJ, Lin CC, Li TC. Glycated Hemoglobin Level and Risk of Hip Fracture in Older People with Type 2 Diabetes: A Competing Risk Analysis of Taiwan Diabetes Cohort Study. *J Bone Miner Res* 2015; **30**: 1338-1346 [PMID: 25598134 DOI: 10.1002/jbmr.2462]
121 **Ntouva A**, Toulis KA, Keerthy D, Adderley NJ, Hanif W, Thayakaran R, Gokhale K, Thomas GN, Khunti K, Tahrani AA, Nirantharakumar K. Hypoglycaemia is associated with increased risk of fractures in patients with type 2 diabetes mellitus: a cohort study. *Eur J Endocrinol* 2019; **180**: 51-58 [PMID: 30400051 DOI: 10.1530/EJE-18-0458]
122 **de Liefde II**, van der Klift M, de Laet CE, van Daele PL, Hofman A, Pols HA. Bone mineral density and fracture risk in type-2 diabetes mellitus: the Rotterdam Study. *Osteoporos Int* 2005; **16**: 1713-1720 [PMID: 15940395 DOI: 10.1007/s00198-005-1909-1]
123 **Ferrari SL**, Abrahamsen B, Napoli N, Akesson K, Chandran M, Eastell R, El-Hajj Fuleihan G, Josse R, Kendler DL, Kraenzlin M, Suzuki A, Pierroz DD, Schwartz AV, Leslie WD; Bone and Diabetes Working Group of IOF. Diagnosis and management of bone fragility in diabetes: an emerging challenge. *Osteoporos Int* 2018; **29**: 2585-2596 [PMID: 30066131 DOI: 10.1007/s00198-018-4650-2]
124 **Leslie WD**, Johansson H, McCloskey EV, Harvey NC, Kanis JA, Hans D. Comparison of Methods for Improving Fracture Risk Assessment in Diabetes: The Manitoba BMD Registry. *J Bone Miner Res* 2018; **33**: 1923-1930 [PMID: 29953670 DOI: 10.1002/jbmr.3538]
125 **Schwartz AV**. Efficacy of Osteoporosis Therapies in Diabetic Patients. *Calcif Tissue Int* 2017; **100**: 165-173 [PMID: 27461216 DOI: 10.1007/s00223-016-0177-8]
126 **Keegan TH**, Schwartz AV, Bauer DC, Sellmeyer DE, Kelsey JL; fracture intervention trial. Effect of alendronate on bone mineral density and biochemical markers of bone turnover in type 2 diabetic women: the fracture intervention trial. *Diabetes Care* 2004; **27**: 1547-1553 [PMID: 15220226 DOI: 10.2337/diacare.27.7.1547]
127 **Inoue D**, Muraoka R, Okazaki R, Nishizawa Y, Sugimoto T. Efficacy and Safety of Risedronate in Osteoporosis Subjects with Comorbid Diabetes, Hypertension, and/or Dyslipidemia: A Post Hoc Analysis of Phase III Trials Conducted in Japan. *Calcif Tissue Int* 2016; **98**: 114-122 [PMID: 26466937 DOI: 10.1007/s00223-015-0071-9]
128 **Vestergaard P**, Rejnmark L, Mosekilde L. Are antiresorptive drugs effective against fractures in patients with diabetes? *Calcif Tissue Int* 2011; **88**: 209-214 [PMID: 21161194 DOI: 10.1007/s00223-010-9450-4]
129 **Abrahamsen B**, Rubin KH, Eiken PA, Eastell R. Characteristics of patients who suffer major osteoporotic fractures despite adhering to alendronate treatment: a National Prescription registry study. *Osteoporos Int* 2013; **24**: 321-328 [PMID: 23070480 DOI: 10.1007/s00198-012-2184-6]
130 **Johnell O**, Kanis JA, Black DM, Balogh A, Poor G, Sarkar S, Zhou C, Pavo I. Associations between baseline risk factors and vertebral fracture risk in the Multiple Outcomes of Raloxifene Evaluation (MORE) Study. *J Bone Miner Res* 2004; **19**: 764-772 [PMID: 15068500 DOI: 10.1359/JBMR.040211]
131 **Ensrud KE**, Stock JL, Barrett-Connor E, Grady D, Mosca L, Khaw KT, Zhao Q, Agnusdei D, Cauley JA. Effects of raloxifene on fracture risk in postmenopausal women: the Raloxifene Use for the Heart Trial. *J Bone Miner Res* 2008; **23**: 112-120 [PMID: 17892376 DOI: 10.1359/jbmr.070904]
132 **Anagnostis P**, Paschou SA, Gkekas NN, Artzouchaltzi AM, Christou K, Stogiannou D, Vryonidou A, Potoupnis M, Goulis DG. Efficacy of anti-osteoporotic medications in patients with type 1 and 2 diabetes mellitus: a systematic review. *Endocrine* 2018; **60**: 373-383 [PMID: 29411304 DOI: 10.1007/s12020-018-1548-x]
133 **Ferrari S**, Eastell R, Napoli N, Schwartz A, Hofbauer LC, Chines A, Wang A, Pannacciulli N, Cummings SR. Denosumab in postmenopausal women with osteoporosis and diabetes: Subgroup

analysis of FREEDOM and FREEDOM extension. *Bone* 2020; **134**: 115268 [PMID: 32058020 DOI: 10.1016/j.bone.2020.115268]

134 **Schwartz AV**, Pavo I, Alam J, Disch DP, Schuster D, Harris JM, Krege JH. Teriparatide in patients with osteoporosis and type 2 diabetes. *Bone* 2016; **91**: 152-158 [PMID: 27374026 DOI: 10.1016/j.bone.2016.06.017]

135 **Liu JM**, Zhu DL, Mu YM, Xia WB; Chinese Society of Osteoporosis and Bone Mineral Research, the Chinese Society of Endocrinology, Chinese Diabetes Society, Chinese Medical Association; Chinese Endocrinologist Association; Chinese Medical Doctor Association. Management of fracture risk in patients with diabetes-Chinese Expert Consensus. *J Diabetes* 2019; **11**: 906-919 [PMID: 31219236 DOI: 10.1111/1753-0407.12962]

136 **Lo JC**, Huang SY, Lee GA, Khandelwal S, Provus J, Ettinger B, Gonzalez JR, Hui RL, Grimsrud CD. Clinical correlates of atypical femoral fracture. *Bone* 2012; **51**: 181-184 [PMID: 22414379 DOI: 10.1016/j.bone.2012.02.632]

137 **Ferron M**, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, Ducy P, Karsenty G. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell* 2010; **142**: 296-308 [PMID: 20655470 DOI: 10.1016/j.cell.2010.06.003]

138 **Schwartz AV**, Schafer AL, Grey A, Vittinghoff E, Palermo L, Lui LY, Wallace RB, Cummings SR, Black DM, Bauer DC, Reid IR. Effects of antiresorptive therapies on glucose metabolism: results from the FIT, HORIZON-PFT, and FREEDOM trials. *J Bone Miner Res* 2013; **28**: 1348-1354 [PMID: 23322676 DOI: 10.1002/jbmr.1865]

139 **Chan DC**, Yang RS, Ho CH, Tsai YS, Wang JJ, Tsai KT. The use of alendronate is associated with a decreased incidence of type 2 diabetes mellitus--a population-based cohort study in Taiwan. *PLoS One* 2015; **10**: e0123279 [PMID: 25874962 DOI: 10.1371/journal.pone.0123279]

140 **Vestergaard P**. Risk of newly diagnosed type 2 diabetes is reduced in users of alendronate. *Calcif Tissue Int* 2011; **89**: 265-270 [PMID: 21710315 DOI: 10.1007/s00223-011-9515-z]

141 **Kiechl S**, Wittmann J, Giaccari A, Knoflach M, Willeit P, Bozec A, Moschen AR, Muscogiuri G, Sorice GP, Kireva T, Summerer M, Wirtz S, Luther J, Mielenz D, Billmeier U, Egger G, Mayr A, Oberhollenzer F, Kronenberg F, Orthofer M, Penninger JM, Meigs JB, Bonora E, Tilg H, Willeit J, Schett G. Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. *Nat Med* 2013; **19**: 358-363 [PMID: 23396210 DOI: 10.1038/nm.3084]

142 **Barrett-Connor E**, Ensrud KE, Harper K, Mason TM, Sashegyi A, Krueger KA, Anderson PW. Post hoc analysis of data from the Multiple Outcomes of Raloxifene Evaluation (MORE) trial on the effects of three years of raloxifene treatment on glycemic control and cardiovascular disease risk factors in women with and without type 2 diabetes. *Clin Ther* 2003; **25**: 919-930 [PMID: 12852708 DOI: 10.1016/s0149-2918(03)80114-5]

143 **Kimura S**, Sasase T, Ohta T, Sato E, Matsushita M. Parathyroid hormone (1-34) improves bone mineral density and glucose metabolism in Spontaneously Diabetic Torii-Lepr(fa) rats. *J Vet Med Sci* 2012; **74**: 103-105 [PMID: 21869570 DOI: 10.1292/jvms.11-0328]

144 **Anastasilakis AD**, Efstathiadou Z, Plevraki E, Koukoulis GN, Slavakis A, Kita M, Avramidis A. Effect of exogenous intermittent recombinant human PTH 1-34 administration and chronic endogenous parathyroid hormone excess on glucose homeostasis and insulin sensitivity. *HormMetab Res* 2008; **40**: 702-707 [PMID: 18553257 DOI: 10.1055/s-2008-1078729]

145 **Celer O**, Akalın A, Oztunali C. Effect of teriparatide treatment on endothelial function, glucose metabolism and inflammation markers in patients with postmenopausal osteoporosis. *Clin Endocrinol (Oxf)* 2016; **85**: 556-560 [PMID: 27321876 DOI: 10.1111/cen.13139]

146 **Paschou SA**, Dede AD, Anagnostis PG, Vryonidou A, Morganstein D, Goulis DG. Type 2 Diabetes and Osteoporosis: A Guide to Optimal Management. *J Clin Endocrinol Metab* 2017; **102**: 3621-3634 [PMID: 28938433 DOI: 10.1210/jc.2017-00042]

147 **Fowlkes JL**, Bunn R C, Thrailkill KM. Contributions of the Insulin/Insulin-Like Growth Factor-1 Axis to Diabetic Osteopathy. *J Diabetes Metab* 2011; **1** [PMID: 23484069 DOI: 10.4172/2155-6156.S1-003]

148 **Liu Y**, Cao Y, Zhang S, Zhang W, Zhang B, Tang Q, Li Z, Wu J. Romosozumab treatment in postmenopausal women with osteoporosis: a meta-analysis of randomized controlled trials. *Climacteric* 2018; **21**: 189-195 [PMID: 29424257 DOI: 10.1080/13697137.2018.1433655]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 730-744

DOI: 10.4239/wjd.v12.i6.730

ISSN 1948-9358 (online)

REVIEW

Mechanisms linking gut microbial metabolites to insulin resistance

Hye Rim Jang, Hui-Young Lee

ORCID number: Hye Rim Jang 0000-0002-8205-1973; Hui-Young Lee 0000-0002-3464-6382.

Author contributions: Jang HR and Lee HY wrote the manuscript; all authors have read and approved the final manuscript.

Supported by National Research Foundation Funded by the Korean Ministry of Science, No. NRF-2018M3A9F3056405 and No. NRF-2020R1A2B5B01002789.

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

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Hye Rim Jang, Hui-Young Lee, Laboratory of Mitochondrial and Metabolic Diseases, Department of Health Sciences and Technology, GAIHST, Gachon University, Incheon 21999, South Korea

Hui-Young Lee, Korea Mouse Metabolic Phenotyping Center, Lee Gil Ya Cancer and Diabetes Institute, Gachon University, Incheon 21999, South Korea

Hui-Young Lee, Division of Molecular Medicine, Department of Medicine, Gachon University College of Medicine, Incheon 21936, South Korea

Corresponding author: Hui-Young Lee, DVM, PhD, Associate Professor, Laboratory of Mitochondrial and Metabolic Diseases, Department of Health Sciences and Technology, GAIHST, Gachon University, 155, Gaetbeol-ro, Yeonsu-gu, Incheon 21999, South Korea. hylee@gachon.ac.kr

Abstract

Insulin resistance is the rate-limiting step in the development of metabolic diseases, including type 2 diabetes. The gut microbiota has been implicated in host energy metabolism and metabolic diseases and is recognized as a quantitatively important organelle in host metabolism, as the human gut harbors 10 trillion bacterial cells. Gut microbiota break down various nutrients and produce metabolites that play fundamental roles in host metabolism and aid in the identification of possible therapeutic targets for metabolic diseases. Therefore, understanding the various effects of bacterial metabolites in the development of insulin resistance is critical. Here, we review the mechanisms linking gut microbial metabolites to insulin resistance in various insulin-responsive tissues.

Key Words: Insulin resistance; Skeletal muscle; Liver; Adipose tissue; Intestine; Gut bacterial metabolites

Core Tip: Since the gut microbiota has been implicated in host energy metabolism and metabolic diseases, understanding mechanisms linked to insulin resistance is a first step in discovery of new drugs and novel targets against metabolic diseases. Here, we review the mechanisms linking gut microbial metabolites to insulin resistance in major target tissues of insulin.

Country/Territory of origin: South Korea

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

Received: February 10, 2021
Peer-review started: February 10, 2021
First decision: March 8, 2021
Revised: March 23, 2021
Accepted: May 20, 2021
Article in press: May 20, 2021
Published online: June 15, 2021

P-Reviewer: Cheng JT, Peng XC
S-Editor: Gao CC
L-Editor: A
P-Editor: Ma YJ

Citation: Jang HR, Lee HY. Mechanisms linking gut microbial metabolites to insulin resistance. *World J Diabetes* 2021; 12(6): 730-744
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/730.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.730

INTRODUCTION

Insulin resistance is a pathological state in which tissues do not respond normally to insulin in the process of glucose metabolism. Insulin is an endocrine hormone that binds to insulin receptors on the plasma membrane of target cells, which induces an anabolic response to nutrient availability[1]. Insulin directly regulates glucose homeostasis by acting on skeletal muscle, the liver, and adipose tissue. These tissues have different functions in metabolic homeostasis that are regulated *via* tissue-specific insulin signaling pathways. In skeletal muscle, insulin stimulates glucose uptake and storage by increasing the expression of glucose transporters (GLUTs) and glycogen synthesis[1,2]. In the liver, insulin activates glycogen synthesis and *de novo* lipogenesis and suppresses gluconeogenesis[1,2]. In adipose tissue, insulin suppresses lipolysis and increases both glucose and fatty acid uptake and lipogenesis[1,2]. In the insulin-resistant state, peripheral glucose disposal is impaired and hepatic gluconeogenesis and adipose lipolysis are not suppressed by insulin. Insulin resistance increases circulating glucose level, which results in increased insulin production in β cells as a compensatory response and hyperinsulinemia, leading to a vicious cycle that promotes further insulin resistance[1,3]. Non-treated and prolonged insulin resistance causes hyperglycemia and type 2 diabetes, and can lead to its complications including hyperlipidemia, metabolic syndrome, nonalcoholic fatty liver disease, and cardiovascular diseases[3,4].

Various factors have been implicated in the pathogenesis of insulin resistance, including genetic predisposition, aging, obesity, and a sedentary lifestyle. More recently, the gut microbiota has been considered to be a key factor leading to the insulin resistance[5]. The gut microbiota regulates host dietary intake, energy metabolism, and energy expenditure[6]. Changes in the composition of the intestinal bacteria might alter energy metabolism and exert various effects on the important metabolic organs, such as skeletal muscle, the liver, and adipose tissue[6]. In addition, the gut microbiota produces thousands of metabolites that accumulate in the gastrointestinal system and can be transferred to distant organs[7]. Lots of recent metabolomics studies examined the association of gut microbiota-derived metabolites with metabolic disease and their effects on host metabolism[8-10]. Therefore, understanding the various effects of bacterial metabolites in the development of insulin resistance becomes critical for discovering novel targets and developing new drugs against metabolic diseases. In this review, we review studies that provide evidence for a relationship between gut bacterial metabolites and insulin resistance, and summarize current mechanisms linking gut microbial metabolites to the development of insulin resistance in various metabolic organs, including skeletal muscle, liver, adipose tissue, and intestine.

EFFECTS OF GUT BACTERIAL METABOLITES ON THE PATHOGENESIS OF INSULIN RESISTANCE

We split this section into four parts for each metabolic organ, and briefly describe the pathophysiology of insulin resistance first followed by further discussions on current mechanisms linking gut microbial metabolites to the development of insulin resistance. The mechanisms for each organ are graphically presented in Figure 1 and the studies for each metabolite are summarized in Table 1.

Skeletal muscle

Skeletal muscle is the primary organ for glucose disposal, accounting for up to 70% of glucose uptake in our body[11]. Insulin promotes glucose uptake in skeletal muscle by translocating the GLUT4 to the plasma membrane[12]. In insulin-sensitive skeletal muscle, the insulin receptor substrate 1-phosphoinositide-3-kinase (PI3K)-AKT arm of the insulin signaling cascade is activated, which increases glucose uptake and glycogen synthesis[1]. In insulin-resistant skeletal muscle, proximal insulin signaling

Table 1 The effects of diet-derived gut bacterial metabolites on the pathogenesis of insulin resistance in various organs

Category	Metabolite	Target organ	Effects	Ref.
Carbohydrate				
Fiber-derived	Acetate	Skeletal muscle	Increased lipid oxidation *in vivo*	Yamashita *et al*[75]
		Liver	Decreased lipogenesis *in vivo*	den Besten *et al*[47] and Yamashita *et al*[51]
			Increased lipid oxidation *in vivo*	den Besten *et al*[47], Yamashita *et al*[51], Kondo *et al*[52] and Sahuri-Arisoylu *et al*[53]
		Adipose tissue	Stimulated adipogenesis *in vitro*	Ge *et al*[60]
			Inhibited lipolysis *in vitro* and *in vivo*	Hong *et al*[59], Ge *et al*[60] and Jocken *et al*[61]
			Increased browning *in vitro* and *in vivo*	Sahuri-Arisoylu *et al*[53] and Hanatani *et al*[73]
		Whole body	Increased energy expenditure and fat oxidation *in vivo* and in humans	den Besten *et al*[47], Canfora *et al*[77] and van der Beek *et al*[78]
	Propionate	Liver	Suppressed gluconeogenesis *in vitro*	Yoshida *et al*[29]
			Decreased lipogenesis *in vivo*	den Besten *et al*[47]
			Increased lipid oxidation *in vivo*	den Besten *et al*[47]
		Adipose tissue	Increased adipogenesis *in vitro*	Ge *et al*[60]
			inhibit lipolysis *in vitro* and *in vivo*	Hong *et al*[59] and Ge *et al*[60]
			Improved inflammation in *ex vivo*	Al-Lahham *et al*[66]
		Intestine	Promoted gluconeogenesis *in vivo*	De Vadder *et al*[91]
		Whole body	Increased energy expenditure and fat oxidation *in vivo* and in humans	den Besten *et al*[47], Canfora *et al*[77] and Chambers *et al*[79]
	Butyrate	Skeletal muscle	Increased lipid oxidation *in vitro* and *in vivo*	Gao *et al*[48]
		Liver	Decreased lipogenesis *in vivo*	den Besten *et al*[47]
			Increased lipid oxidation *in vivo*	den Besten *et al*[47], Gao *et al*[48] and Mollica *et al*[49]
		Adipose tissue	decreased lipolysis *in vitro*	Ohira *et al*[67]
			Improved inflammation *in vitro*	Ohira *et al*[67]
			Increased thermogenesis *in vivo*	Gao *et al*[48] and Li *et al*[74]
		Intestine	Promoted gluconeogenesis *in vitro* and *in vivo*	De Vadder *et al*[91]
		Whole body	Increased energy expenditure and fat oxidation *in vivo* and in humans	den Besten *et al*[47], Gao *et al*[48] and Canfora *et al*[77]
	Succinate	Intestine	Promoted gluconeogenesis *in vivo*	De Vadder *et al*[92]
Protein				
Protein-derived	Hydrogen sulfide	Liver	Increased gluconeogenesis *in vitro*	Zhang *et al*[32]
			Decreased glycogen synthesis *in vitro*	Zhang *et al*[32]
	Indole	Adipose	Increased inflammation *in vivo*	Virtue *et al*[10]

		tissue		
	Indole-3-carboxylic acid	Adipose tissue	Increased inflammation *in vivo*	Virtue *et al*[10]
	Phenylacetic acid	Liver	Increased lipogenesis in *ex vivo* and *in vivo*	Hoyles *et al*[46]
Lipid and others				
Linoleic acid-derived	10-oxo-12(Z)-octadecenoic acid	Adipose tissue	Induced adipogenesis *in vitro*	Goto *et al*[55]
			Increased thermogenesis *in vivo*	Kim *et al*[81]
	Conjugated linoleic acid	Adipose tissue	Increased energy expenditure	Takahashi *et al*[82], Park *et al*[83] and Lee *et al*[84]
Ferulic acid-derived	Ferulic acid 4-O-sulfate and Dihydroferulic acid 4-O-sulfate	Skeletal muscle	Increased glucose uptake *in vitro*	Houghton *et al*[19]
Resveratrol-derived	*Trans*-resveratrol 4'-O-glucuro-nide and *Trans*-resveratrol 3-O-sulfate	Skeletal muscle	Increased glucose uptake *in vitro*	Houghton *et al*[19]
Berries-derived	Isovanillic acid 3-O-sulfate	Skeletal muscle	Increased glucose uptake *in vitro*	Houghton *et al*[19]
Catecin-derived	4-hydroxy-5-(3,4,5-trihydroxyphenyl) valeric acid, 5-(3,4,5-trihydroxyphenyl)-γ-valerolac-tone, and 5-(3-hydroxyphenyl) valeric acid	Skeletal muscle	Increased glucose uptake *in vitro*	Takagaki *et al*[22]
Catecin-derived	5-(3,5-dihydroxyphenyl)-γ-valerolactone	Skeletal muscle	Increased glucose uptake *in vitro* and *in vivo*	Takagaki *et al*[22]
Bacteria-derived	Extracellular vesicles	Skeletal muscle	Decreased glucose uptake *in vivo*	Choi *et al*[20]
Choline-derived	Trimethylamine N-oxide	Liver	Increased gluconeogenesis in *ex vivo* and *in vivo*	Chen *et al*[33] and Gao *et al*[43]
		Adipose tissue	Promoted inflammation *in vivo*	Gao *et al*[43]

events are impaired, which blocks the function of insulin to translocate GLUT4 to plasma membrane and to stimulate glycogen synthesis[1]. Furthermore, when calorie loads exceed the glucose uptake capacity of skeletal muscle, the circulating glucose mostly returns to the liver, triggering hepatic *de novo* lipogenesis[3], which causes ectopic fat deposition in the liver and other tissues, further exacerbating insulin resistance[13]. Therefore, impaired glucose uptake in skeletal muscle has been considered as a major culprit of type 2 diabetes[14-16] and targeted as a therapeutic strategy against insulin resistance[17,18].

A recent study suggests that microbial products derived from phenolic acids may increase glucose uptake in skeletal muscle under insulin-stimulated condition[19]. Microbiota-produced phenolic metabolites are derived from ferulic acid, resveratrol, and berries. The ferulic acid-derived metabolites, ferulic acid 4-O-sulfate and dihydroferulic acid 4-O-sulfate, and the resveratrol-derived metabolites, *trans*-resveratrol 4'-O-glucuronide and *trans*-resveratrol 3-O-sulfate, increased 2-deoxy-D-[1-^{14}C(U)]-glucose uptake in LHCN-M2 human skeletal muscle cells[19]. Isovanillic acid 3-O-sulfate, which is primarily derived from berries, increased glucose uptake in myotubes through GLUT4-PI3K-AKT-dependent mechanisms and stimulated dose-dependent glucose uptake[19]. On the other hands, a study has been shown that bacterial-derived metabolite-complex can decrease glucose uptake, though the exact composition of the complex is not defined[20]. Gut bacteria-derived extracellular vesicles (EVs), which are phospholipid spherical bilayer, are ubiquitously produced by gram-negative bacteria [20]. Especially, *Pseudomonas panacis*-derived EVs increasing in a high-fat diet (HFD)-fed mice compared to regular chow-fed mice as well as gut microbe-derived EVs from HFD-fed mice stools induced insulin resistance, including impairment of insulin signaling both *in vitro* and *in vivo,* and impairment of glucose uptake by decreasing insulin-dependent GLUT4 translocation, both in myotubes and adipocytes [20].

Insulin-independent glucose uptake is also activated by microbial metabolites. Activation of AMP-activated protein kinase (AMPK) in response to exercise regulates the translocation of GLUT4 storage vesicles and promotes insulin-independent glucose uptake[21]. In particular, 5-(3,5-dihydroxyphenyl)-γ-valerolactone has been shown to increase GLUT4 translocation *via* activation of AMPK through an insulin-independent pathway in skeletal muscle both *in vitro* and *in vivo*[22]. The antidiabetic green tea

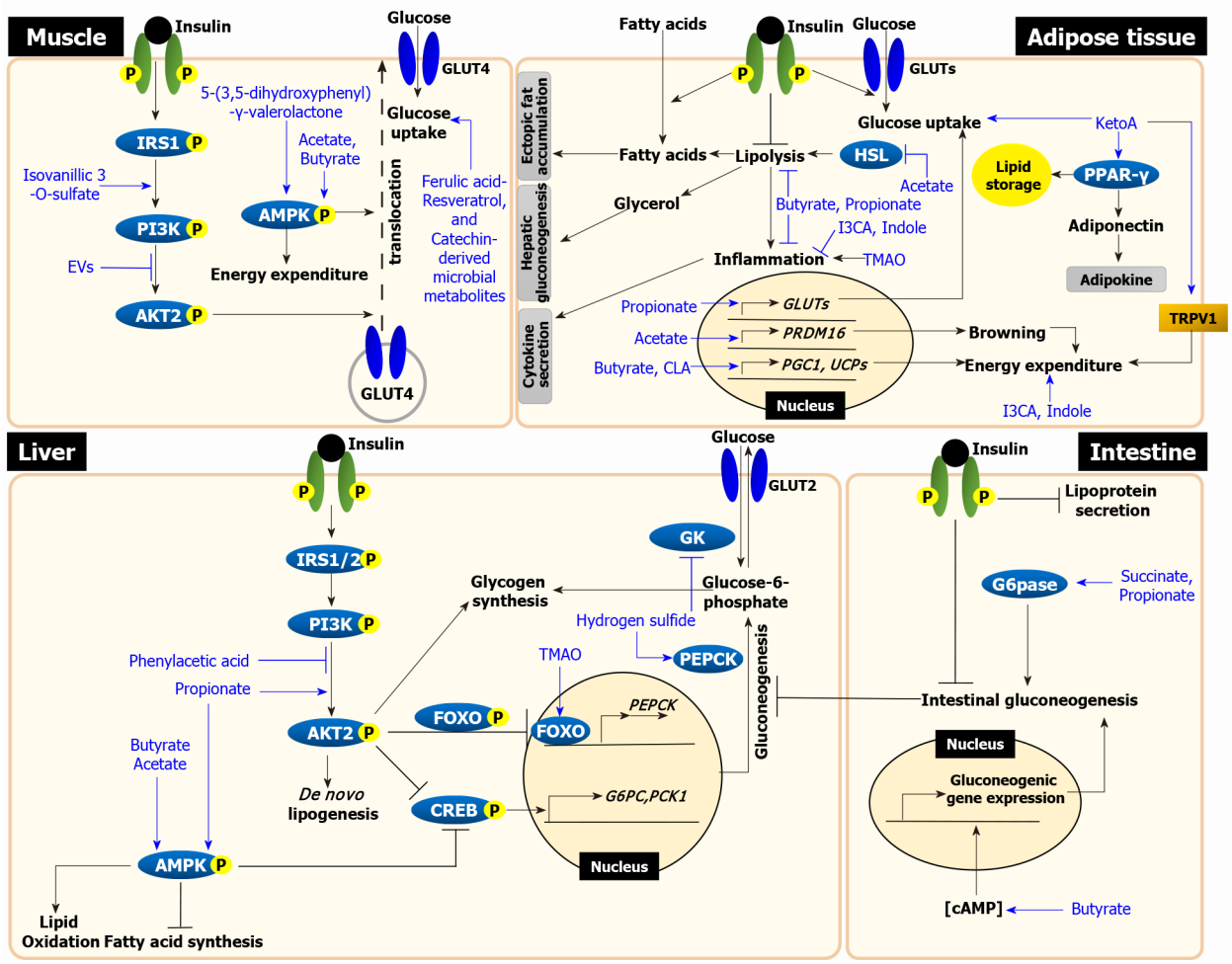

Figure 1 The mechanisms linking microbial metabolites to insulin resistance. In skeletal muscle, insulin stimulates glucose uptake by translocating glucose transporter (GLUT) 4 *via* insulin receptor substrate (IRS)-phosphoinositide-3-kinase (PI3K)-AKT signaling. Isovanillic 3-O-sulfate increases glucose uptake by activating PI3K-AKT pathway. Gut bacteria-derived extracellular vesicles (EVs) decrease glucose uptake by inhibiting AKT phosphorylation. Glucose uptake is also increased *via* AMP-activated protein kinase (AMPK) activation, insulin-independent. 5-(3,5-dihydroxyphenyl-γ-valerolactone activates AMPK phosphorylation, which enhances glucose uptake. Although mechanisms have been unknown, other ferulic acid, resveratrol, and catechin-derived microbial metabolites also enhance glucose uptake (left upper panel). In liver, insulin activates glycogen synthesis and *de novo* lipogenesis and suppresses gluconeogenesis *via* IRS-PI3K-AKT signaling. Propionate increases the phosphorylation of both AKT and AMPK, which suppresses gluconeogenesis. Hydrogen sulfide stimulates gluconeogenesis *via* phosphoenolpyruvate carboxykinase activation and reduces glycogen synthesis *via* the inhibition of glucokinase activity. Trimethylamine N-oxide (TMAO) increases gluconeogenesis *via* PKR-like ER kinase-FOXO1 pathway. Phenylacetic acid inhibits AKT phosphorylation. All short chain fatty acids (SCFAs), including acetate, propionate, and butyrate, activate AMPK phosphorylation, which lead to decrease lipid accumulation (left lower panel). In adipose tissue, insulin stimulates glucose and fatty acid uptake and suppress lipolysis. Failure to suppress lipolysis in insulin-resistant adipose tissue increases circulating free fatty acids and glycerol, which leads to an increase in ectopic fat accumulation in the liver and muscle and stimulates hepatic gluconeogenesis. 10-oxo-12(Z)-octadecenoic acid (KetoA) increases insulin-stimulated glucose uptake and energy expenditure *via* TRPV2 activation. KetoA also increases the production and secretion of adiponectin *via* peroxisome proliferator-activated receptor-γ activation. TMAO increases inflammation in adipocyte. Indole and I3CA have anti-inflammatory effects. Conjugated linoleic acid enhances energy expenditure by increasing the expression of uncoupling proteins (UCPs) genes. All SCFAs inhibit lipolysis. Acetate inhibits lipolysis by suppressing HSL and stimulates also browning by increasing the expression of browning-related genes. Butyrate and propionate attenuate inflammation. Propionate increases glucose uptake by increasing GLUT4 expression. Butyrate enhances energy expenditure by upregulating PPAR-γ coactivator 1 and UCPs genes (right upper panel). The intestine, as discussed in this review, is an organ that actively interacts with gut bacteria and accumulates microbial metabolites. Intestinal lipoprotein secretion and gluconeogenesis are suppressed by insulin. In intestine, propionate and succinate act as gluconeogenic substrate, which activate gluconeogenesis *via* G6Pase activation. Butyrate increases cyclic adenosine monophosphate levels, which upregulates the expression of gluconeogenic genes and increases gluconeogenesis. Through this mechanisms, increased intestinal gluconeogenesis suppresses hepatic gluconeogenesis (right lower panel). Black lines represent insulin resistance-related events and blue lines represent action of metabolites. Grey boxes represent the effects of adipose tissue on other tissues. EVs: Extracellular vesicles; PEPCK: Phosphoenolpyruvate carboxykinase; GK: Glucokinase; TMAO: Trimethylamine N-oxide; PERK: PKR-like ER kinase; FOXO1: Forkhead box protein O1; cAMP: Cyclic adenosine monophosphate; CREB: cAMP-response element binding protein; G6PC: Glucose 6-phosphatase catalytic subunit; PCK1: Phosphoenolpyruvate carboxykinase 1; TRPV1: Transient receptor potential vanilloid 1; PPAR: Peroxisome proliferator-activated receptor; I3CA: Indole-3-carboxylic acid; CLA: Conjugated linoleic acid; HSL: Hormone-sensitive lipase; PRDM16: PR domain containing 16; PGC1: Peroxisome proliferator-activated receptor-gamma coactivator 1; G6Pase: Glucose 6-phosphatase.

catechin (-)-epigallocatechin gallate (EGCG) is further degraded by *Flavonifractor plautii*[23], and several gut bacteria-derived EGCG metabolites, including 5-(3,5-dihydroxyphenyl)-γ-valerolactone, 4-hydroxy-5-(3,4,5-trihydroxyphenyl)valeric acid,

5-(3,4,5-trihydroxyphenyl)-γ-valerolactone, and 5-(3-hydroxyphenyl) valeric acid, have been shown to promote 2-deoxy-glucose uptake in myotubes *in vitro*[22]. It has been reported that *Flavonifractor plautii* was decreased in fecal microbiota of subjects with mild fasting hyperglycemia[24].

Liver

The liver is a central organ that coordinates whole-body metabolism, including carbohydrate, lipid, and protein metabolism. The liver is responsible for gluconeogenesis, glycogenolysis, glycogen synthesis, and *de novo* lipogenesis[1]. In contrast to skeletal muscle, hepatic glucose uptake is not regulated by insulin but blood glucose levels because GLUT2, a transporter with a high K_M for glucose, is abundantly expressed in the liver and not translocated by insulin stimulation[25]. Rather than regulating the glucose uptake, in the liver, insulin suppresses hepatic glucose production by reducing the transcription of gluconeogenic enzymes[26,27] and induces a shift from net glucose production to net glucose storage by simultaneous regulation of glycogenolysis and glycogen synthesis[2]. However, in an insulin-resistant state, these regulations are not controlled by insulin, and the non-suppressed hepatic glucose production under insulin stimulated condition has been considered as a maker for hepatic insulin resistance[1,28]. It has been reported that propionate, a gut microbial product derived from carbohydrate fermentation, regulates hepatic gluconeogenesis under insulin stimulated condition[29]. Previously, stable isotope studies in both humans and animals have showed that propionate is used as a gluconeogenic substrate in the liver rather than being directly oxidized[30,31]. Recently, it was reported that propionate effectively suppresses hepatic glucose production in both presence and absence of long chain fatty acid by increasing the expression of gluconeogenesis-related genes, including *G6PC* and *PCK1*, *via* the G protein-coupled receptor (GPCR) 43-mediataed AMPK signaling pathway under insulin-stimulated condition as well as increases AKT phosphorylation in HepG2 hepatocyte[29]. In addition to insulin-stimulated condition, it has been reported that gut bacterial metabolites regulate hepatic glucose production under non-insulin stimulated basal condition [32, 33]. Hydrogen sulfide, a product of protein fermentation, is not only generated in the body but also produced by sulfate-reducing bacteria, including *Desulfovibrio*, *Desulfobacter*, *Desulfomonas*, and *Desulfobulbus*, in the colon[32,34] and affects the basal hepatic glucose production. Under basal condition, this metabolite impairs glucose homeostasis by stimulating gluconeogenesis *via* increased phosphoenolpyruvate carboxykinase activity and decreased glucokinase by reducing glycogen synthesis in HepG2 human hepatoma cells[32]. In type 2 diabetes patients, it has been reported that the plasma hydrogen sulfide levels were reduced compared to healthy subjects[35,36], suggesting clinical association of microbial metabolites in hypergly-cemia. Trimethylamine N-oxide (TMAO), which is known as a gut bacterial metabolite derived from choline, is converted by hepatic enzymes from trimethylamine, a choline-derived microbial metabolite, in liver[37]. The production of TMAO is completely suppressed in both antibiotics-treated humans and mice but the plasma of TMAO levels return to normal after the withdrawal of the antibiotics[38,39]. It has been reported that TMAO increases with insulin resistance in both humans and animals[40-42]. In mice, TMAO treatment promoted glucose intolerance, while a reduction of TMAO prevented glucose intolerance[33]. Under basal condition, the treatment of TMAO in mice activated PKR-like ER kinase and increased gluconeogenic gene expression, including *G6pc* and *Pck1*, *via* the forkhead box protein O1 transcription factor, which promoted hyperglycemia[33,43].

In addition to glucose metabolism, insulin also controls lipid metabolism in the liver. Since insulin normally promotes net hepatic *de novo* lipogenesis, one might expect decreased *de novo* lipogenesis in an insulin-resistant state; however, hepatic insulin resistance is highly associated with hepatic steatosis[1,3], and *de novo* lipogenesis is consistently elevated in insulin-resistant liver tissue[3]. This phenomenon has been termed "selective hepatic insulin resistance," as glucose metabo-lism is affected by insulin resistance but lipid metabolism is not affected[44]. The increased *de novo* lipogenesis could be partly accounted by hyperinsulinemia, but still there are selective insulin resistance between glucose and lipid when considers the action of insulin *per se*[3,4]. Nevertheless, microbial metabolites can regulate the hepatic lipid metabolism. Phenylacetic acid is a microbial metabolite derived from aromatic compounds and produced by *Bacteroides spp.*, which have aromatic amino acids fermentative activities[45]. Plasma phenylacetic acid positively correlates with the nonalcoholic fatty liver disease activity score in humans[9,46]. Phenylacetic acid induced the accumulation of hepatic triglycerides both in cellular and animal studies [46]. The metabolite also reduced insulin-induced AKT phosphorylation in human

primary hepatocytes[46]. In contrast, short chain fatty acids (SCFAs), including acetate, propionate, and butyrate, decreased hepatic lipid accumulation. Administration of all three SCFAs in HFD-fed mice decreased not only total body fat content, without a change in food intake, but also the expression of genes related to hepatic lipogenesis and fatty acid synthase[47]. In addition, hepatic lipid oxidation capacity in SCFA-fed mice was increased *via* upregulation of mitochondrial uncoupling protein (UCP) 2 expression and activation of AMPK[47-49]. The SCFA acetate inhibited fatty acid synthesis in the liver *via* activation of AMPK. Oral administration of acetate stimulated the phosphorylation of AMPK, which inactivates carbohydrate-responsive element-binding protein[50], and in turn modulated the transcription of lipogenic genes in the liver[51]. Acetate also suppressed the increases in whole-body fat mass and hepatic lipid accumulation by increasing the expression of genes encoding peroxisome proliferator-activated receptor (PPAR) α and fatty acid oxidation-related proteins through AMPK α2 in the liver[52]. In mice, acetate treatment improved liver mitochondrial function by increasing the number of cristae, the location of the electron transport chain, per mitochondria, and the expression of complexes III, IV, and V[53]. Another SCFA, butyrate, increased mitochondrial mass and area and improved fatty acid oxidation in the liver of HFD-fed mice[49].

Adipose tissue

Adipose tissue is an energy storage organ[5]. In adipose tissue, insulin-stimulated glucose uptake also occurs *via* GLUT4 translocation, which is greatly reduced in insulin resistant condition, such as obesity and type 2 diabetes[54]. A linoleic acid-derived fatty acid generated by gut lactic acid bacteria, 10-oxo-12(Z)-octadecenoic acid (KetoA), induced adipocyte differentiation *via* activation of PPARγ, and increased the production of adiponectin and insulin-stimulated glucose uptake in 3T3-L1 murine adipocytes[55]. However, physiologically, adipose tissue is not quantitatively significant in insulin-stimulated glucose disposal because it accounts for < 5% of blood glucose uptake in our body[16]. Rather than glucose metabolism, insulin may have more critical roles in lipid metabolism of adipose tissues, thus the suppression of lipolysis is an important function of insulin in adipose tissue[4]. Failure to suppress lipolysis in insulin-resistant adipose tissue increases circulating free fatty acids and glycerol[15,56], and affects in hepatic glucose production[1]. These increased levels of circulating free fatty acids lead to an increase in ectopic fat accumulation in the liver and muscle, further exacerbating insulin resistance[15]. In addition, the glycerol released from adipose tissue serves as a gluconeogenic substrate and stimulates hepatic gluconeogenesis[1]. Suppression of lipolysis also reduces the levels of acetyl-CoA, an allosteric activator of pyruvate carboxylase, decreases pyruvate carboxylase activity[57]. As a result, gluconeogenic flux, involving glycerol and acetyl-CoA, is diminished, resulting in decreased hepatic gluconeogenesis[57]. Therefore, the regulation of lipolysis in adipose tissue is considered a therapeutic strategy against insulin resistance[58]. In the SCFAs, it has been reported both acetate and propionate stimulate adipogenesis and inhibit lipolysis *via* activation of GPCR43 but not GPCR41 [59,60]. Acetate might inhibit basal and beta-adrenergic receptor-mediated intracellular lipolysis *via* attenuation of hormone-sensitive lipase phosphorylation in human adipose tissue-derived adipocytes and lead to a reduction in non-esterified fatty acid release[61]. Injection of acetate into fasted mice led to decreased plasma free fatty acid levels *via* activation of GPCR43[60].

Adipose tissue also functions as an endocrine organ and releases adipokines, lipids, and cytokines, which regulates whole-body metabolism[62]. Adipose tissue can secrete molecules associated with improved insulin sensitivity, including adiponectin and branched fatty acid esters of hydroxyl fatty acids[63]. Chronic low-grade inflammation occurs in obese individuals with insulin resistance, which is mainly induced by adipose tissue inflammation[64]. Inflammation of adipose tissue is caused by macrophage infiltration, which impairs the insulin sensitivity of insulin target organs, resulting in insulin resistance[65]. TMAO, a microbial metabolite derived from choline, promoted adipose tissue inflammation in HFD-fed mice by increasing mRNA and serum levels of monocyte chemoattractant protein-1 (MCP-1), the proinflammatory cytokine, and decreasing mRNA and serum levels of interleukin (IL)-10, the anti-inflammatory cytokine, in adipose tissue[43]. Conversely, the SCFAs propionate and butyrate improved adipose tissue inflammation. Propionate may have a directly beneficial effect on adipose tissue in overweight subjects, as it reduced the mRNA expression and secretion of inflammatory cytokines and increased the mRNA expression of genes involved in lipogenesis (*e.g.*, *LPL, SREBP1c)* and glucose uptake (*e.g.*, GLUT4)[66]. Butyrate suppressed lipolysis and inflammatory responses, including the upregulation of tumor necrosis factor-α, MCP-1, and IL-6, which are generated by

the interaction of adipocytes and macrophages [67]. It has been reported that gut bacterial metabolites derived from protein fermentation have anti-inflammatory effects in adipose tissue. Indole-3-carboxylic acid and indole, a tryptophan-derived microbial metabolites, are decreased in the cecal contents of HFD-fed mice compared to regular chow-fed mice[10]. These metabolites increased energy expenditure and improved insulin sensitivity by decreasing the expression of the *microRNA miR-181*, which is upregulated in HFD feeding and increases white adipose tissue (WAT) inflammation[10].

Brown adipose tissue and whole body energy expenditure

Unlike WAT, because brown adipose tissue (BAT) is responsible for energy expenditure by burning fatty acids to produce heat, it is an important organ that effects on whole body energy metabolism[68]. Similarly increasing beige adipocytes in WAT, a process termed "browning," results in increased heat production and energy expenditure[69]. Therefore, enhanced BAT activity and browning of WAT are important for energy expenditure and are thought to influence insulin sensi-tivity[68, 69]. It has been reported that gut bacterial metabolites derived from carbohydrates and fatty acids increase energy expenditure *via* browning of WAT and/or enhancing the function of BAT. SCFAs, including acetate, propionate, and butyrate, which are products of dietary fiber fermentation by gut bacteria. Acetate is mainly produced by *Bifidobacteria* and *Lactobacillus* and propionate is largely produced by *Bacteroides and Veillonella*, such as *Bacteroides eggerthii, Bacteroides fragilis,* and *Veillonella parvula*[70,71]. Butyrate is mostly produced by anaerobic bacteria, including *Faecalibacterium prausnitzii,* and *Eubacterium rectale*[72]. Acetate enhanced beige fat differentiation of white adipocytes *in vitro*[73]. Acetate and butyrate promoted browning in adipocytes [53,74]. In obese diabetic mice, acetate also induced browning of adipocytes by increasing thermogenesis-related gene expression, altered adipocyte morphology, and increased the thermogenic capacity of adipose tissue, independent of BAT, in HFD-fed mice[53,73]. Butyrate exerted an anti-obesity effect in animal models by strengthening the function of BAT. This anti-obesity effect occurs by increasing in energy expenditure and fat oxidation through upregulation of the expression of thermo-genesis-related genes in BAT, such as PPAR-γ coactivator 1-α (PGC1α) and UCP1[48, 74]. All SCFAs stimulated lipid oxidation by activating AMPK in the liver and adipose tissue[47]. Similarly, in skeletal muscle, acetate improved oxygen consumption by increasing the expression of lipid oxidation-related genes and AMPK activity in animal models[75]. Butyrate increased oxygen consumption and energy expenditure both *in vitro* and *in vivo*. These effects are caused by activation of AMPK and inhibition of histone deacetylases, which activate PGC1α and subsequently increase the expression of PPARδ, thus promoting fatty acid oxidation and increasing the proportion of type I muscle fibers, which are characterized by their high oxidative capacity[48]. In contrast to animal models, treatment of both lean and metabolic syndrome subjects with butyrate had no effect on BAT function[76], and open to debate over the single treatment. However, infusion of SCFA mixtures of acetate, propionate, and butyrate increased fat oxidation and whole-body energy expenditure in overweight/obese men[77,78]. These effects were observed following treatment with each SCFA alone as well as mixtures of the SCFAs. Propionate increased whole-body energy expenditure and fat oxidation in healthy and overweight/obese humans [79]. These findings suggested that SCFAs affect whole-body energy expenditure through a combination of mechanisms in different tissues, including skeletal muscle, the liver, and adipose tissue.

There are several studies show that fatty acid-derived microbial metabolites effect on energy expenditure. KetoA, a linoleic acid-derived microbial metabolite, has been suggested as the regulator of host energy metabolism. The anti-obesity effect of KetoA is shown *via* the activation of transient receptor potential vanilloid 1 (TRPV1), a member of the TRPV channel family, which has been reported to be important for the regulation of energy metabolism in adipocytes[80,81]. KetoA-induced TRPV1 activation enhanced energy expenditure by increasing the function of both BAT and WAT in diabetic mice[81]. Conjugated linoleic acid (CLA) is also mainly produced from linoleic acid by lactic acid bacteria, and enhances energy expenditure *via* increase in the expression of UCPs genes in adipose tissue[82,83]. Mice fed CLA-producing bacteria, *Lactobacillus rhamnosus* PL60, are prevented from diet-induced obesity and hepatic steatosis[84].

Intestine

The small intestine takes up glucose from the intestinal lumen mainly through sodium-glucose cotransporter 1 and transports glucose from enterocytes to the blood

via GLUT2[85]. Although it has been reported that insulin inhibits the translocation of GLUT2 from the basolateral surface to the apical epithelial membrane, the role of insulin in intestinal glucose uptake is unclear[85,86]. Insulin signaling has been implicated in both increased and decreased glucose uptake from the intestinal lumen to the enterocytes in both humans and *in vitro* studies[87]. Beside the glucose uptake, insulin's action on lipid metabolism and gluconeogenesis seems to be well established in intestine. Insulin modulates lipoprotein metabolism in the intestine and suppresses lipoprotein secretion[86]. Production of apolipoproteinB48-containing chylomicrons by the small intestine increases insulin resistance[86,88]. Like liver, the intestine shows gluconeogenic capacity. Intestinal glucose production is suppressed by insulin and increased in insulinopenic states, such as a 48-h fasting and type 1 diabetes[89,90]. In a postprandial state, intestinal gluconeogenesis (IGN) accounts for about 5%-7% of the total endogenous glucose production[91-93]. A protein-rich diet increases the expression of genes involved in IGN in the intestine and the regulatory enzymes in gluconeogenesis and glutaminase[94]. In intestine, the peptides digested from protein-rich diet act on μ-opioide receptors presenting in the portal vein nerves[94], which signals to the brain and releases of the neuromediator vasoactive intestinal peptide from brain during the postprandial period[95]. This neuromediator activates adenylate cyclase and increases cAMP levels, which induces the expression of IGN-related genes [95]. The IGN-related enzymes are progressively induced during the postprandial period, and the amount of enzyme is maintained during the post-absorptive period[94, 96]. In addition, because protein-rich diets provide major IGN substrates, including glutamine and glutamate, these substrates can be utilized by IGN induced during the post-absorptive period[97]. It was recently reported that IGN protects against diabetes and obesity by suppressing hepatic gluconeogenesis and positively regulating glucose homeostasis[91,92]. Gastric bypass surgery also has been reported to increase IGN and suppress hepatic gluconeogenesis in diabetic animal model[93,98].

It has been reported that IGN is induced by the SCFAs and succinate, the gut microbial metabolites derived from carbohydrate fermentation[91,92]. The SCFA propionate, as a gluconeogenic substrate, activated G6Pase activity and increased IGN gene expression *via* vasoactive intestinal peptide released from brain through a gut-brain neural circuit involving the free fatty acid receptor 3-dependent stimulation [91, 95]. Propionate showed the strongest capacity to induce intestinal glucose production among SCFAs[91]. Another SCFA, butyrate increased the levels of ATP, a substrate of adenylate cyclase, which promoted the production of cyclic AMP[91]. Cyclic AMP functions as an intracellular messenger that stimulates the expression of genes involved in gluconeogenesis[99]. Through this mechanism, butyrate promoted gluconeogenesis in enterocytes[91]. Microbial-derived succinate not only showed an anti-obesity effect but also improved glucose tolerance and insulin sensitivity. Succinate functioned as a gluconeogenic substrate such as propionate, and it was shown to promote activation of gluconeogenesis in the intestine of high-fat and high-sucrose diet-fed mice[92]. In addition, succinate-fed wild-type mice showed a decreased capacity for hepatic glucose production, and this suppression of hepatic gluconeogenesis was absent in succinate-fed *G6pc* intestinal-specific knockout mice[92]. In humans, it has been reported that succinate-producing bacteria, including *Bacteroidaceae* and *Prevotella*, were found to be increased in fecal samples of patients with non-alcoholic steatohepatitis[92,100].

CONCLUSION

Since the association between microbiome and metabolic diseases in the last 20 years has been increasingly revealed, microbial metabolites are considered to be the link between microbiome and metabolic diseases. This review summarized the role of microbial metabolites in the major mechanisms representing insulin resistance in each tissue. Through this, in addition to SCFAs, which has been studied a lot in the past, recent studies have found some candidates that protein-derived (*e.g.*, hydrogen sulfide, Indole-3-carboxylic acid, and phenylacetic acid) and lipid-derived microbial metabolites (*e.g.*, KetoA and CLA) can play a role in the pathogenesis of insulin resistance. However, metabolites by gut bacteria are highly diverse depending on intestinal environments (*e.g.*, dietary substrates, host enzyme, acidity, temperature, and antibiotics), yet only limited number of metabolites have been identified and functionally studied in metabolic diseases. Indeed, according to Human Microbiome Database, over 100 thousand of metabolites are exited in our body, but only hundreds are counted as bacterial-specific (https://hmdb.ca/statistics).

What makes this even more challengeable is the complex etiology of insulin resistance. In glucose and lipid metabolism, each organ is highly interrelated. Muscle insulin resistance can divert ingested glucose into the liver, and increase hepatic de novo lipogenesis and gluconeogenesis. Adipose tissue insulin resistance can release lipogenic and gluconeogenic substrates to liver as well intestinal IGN reversely controls the hepatic gluconeogenesis. In order to develop bacterial metabolites as a therapeutic agent for insulin resistance in humans, not only clarifying the exact mechanism of action for which stage of insulin resistance, but also understanding metabolic complexities between multiple organs should be conducted in parallel. Nevertheless, insulin resistance is a common prerequisite for various metabolic diseases, the discovery of metabolites that specifically act on insulin resistance is a strategy to overcome metabolic diseases in terms of more fundamental etiology and early prevention, and more research should be conducted.

REFERENCES

1 **Petersen MC**, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. *Physiol Rev* 2018; **98**: 2133-2223 [PMID: 30067154 DOI: 10.1152/physrev.00063.2017]

2 **Samuel VT**, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell* 2012; **148**: 852-871 [PMID: 22385956 DOI: 10.1016/j.cell.2012.02.017]

3 **Samuel VT**, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest* 2016; **126**: 12-22 [PMID: 26727229 DOI: 10.1172/JCI77812]

4 **Erion DM**, Park HJ, Lee HY. The role of lipids in the pathogenesis and treatment of type 2 diabetes and associated co-morbidities. *BMB Rep* 2016; **49**: 139-148 [PMID: 26728273 DOI: 10.5483/bmbrep.2016.49.3.268]

5 **Gesta S**, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. *Cell* 2007; **131**: 242-256 [PMID: 17956727 DOI: 10.1016/j.cell.2007.10.004]

6 **Lee Y**, Lee HY. Revisiting the Bacterial Phylum Composition in Metabolic Diseases Focused on Host Energy Metabolism. *Diabetes Metab J* 2020; **44**: 658-667 [PMID: 32662252 DOI: 10.4093/dmj.2019.0220]

7 **Schroeder BO**, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med* 2016; **22**: 1079-1089 [PMID: 27711063 DOI: 10.1038/nm.4185]

8 **Chen MX**, Wang SY, Kuo CH, Tsai IL. Metabolome analysis for investigating host-gut microbiota interactions. *J Formos Med Assoc* 2019; **118** Suppl 1: S10-S22 [PMID: 30269936 DOI: 10.1016/j.jfma.2018.09.007]

9 **Wikoff WR**, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA* 2009; **106**: 3698-3703 [PMID: 19234110 DOI: 10.1073/pnas.0812874106]

10 **Virtue AT**, McCright SJ, Wright JM, Jimenez MT, Mowel WK, Kotzin JJ, Joannas L, Basavappa MG, Spencer SP, Clark ML, Eisennagel SH, Williams A, Levy M, Manne S, Henrickson SE, Wherry EJ, Thaiss CA, Elinav E, Henao-Mejia J. The gut microbiota regulates white adipose tissue inflammation and obesity *via* a family of microRNAs. *Sci Transl Med* 2019; **11** [PMID: 31189717 DOI: 10.1126/scitranslmed.aav1892]

11 **Baron AD**, Brechtel G, Wallace P, Edelman SV. Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol* 1988; **255**: E769-E774 [PMID: 3059816 DOI: 10.1152/ajpendo.1988.255.6.E769]

12 **Zorzano A**, Muñoz P, Camps M, Mora C, Testar X, Palacín M. Insulin-induced redistribution of GLUT4 glucose carriers in the muscle fiber. In search of GLUT4 trafficking pathways. *Diabetes* 1996; **45** Suppl 1: S70-S81 [PMID: 8529804 DOI: 10.2337/diab.45.1.s70]

13 **Perry RJ**, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature* 2014; **510**: 84-91 [PMID: 24899308 DOI: 10.1038/nature13478]

14 **DeFronzo RA**, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 2009; **32** Suppl 2: S157-S163 [PMID: 19875544 DOI: 10.2337/dc09-S302]

15 **Shulman GI**. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med* 2014; **371**: 1131-1141 [PMID: 25229917 DOI: 10.1056/NEJMra1011035]

16 **Kowalski GM**, Bruce CR. The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents. *Am J Physiol Endocrinol Metab* 2014; **307**: E859-E871 [PMID: 25205823 DOI: 10.1152/ajpendo.00165.2014]

17 **Kalinovich A**, Dehvari N, Åslund A, van Beek S, Halleskog C, Olsen J, Forsberg E, Zacharewicz E, Schaart G, Rinde M, Sandström A, Berlin R, Östenson CG, Hoeks J, Bengtsson T. Treatment with a β-2-adrenoceptor agonist stimulates glucose uptake in skeletal muscle and improves glucose homeostasis, insulin resistance and hepatic steatosis in mice with diet-induced obesity. *Diabetologia* 2020; **63**: 1603-1615 [PMID: 32472192 DOI: 10.1007/s00125-020-05171-y]

18 **Vieira R**, Souto SB, Sánchez-López E, Machado AL, Severino P, Jose S, Santini A, Fortuna A, García ML, Silva AM, Souto EB. Sugar-Lowering Drugs for Type 2 Diabetes Mellitus and Metabolic Syndrome-Review of Classical and New Compounds: Part-I. *Pharmaceuticals (Basel)*

2019; **12** [PMID: 31658729 DOI: 10.3390/ph12040152]
19 **Houghton MJ**, Kerimi A, Mouly V, Tumova S, Williamson G. Gut microbiome catabolites as novel modulators of muscle cell glucose metabolism. *FASEB J* 2019; **33**: 1887-1898 [PMID: 30183376 DOI: 10.1096/fj.201801209R]
20 **Choi Y**, Kwon Y, Kim DK, Jeon J, Jang SC, Wang T, Ban M, Kim MH, Jeon SG, Kim MS, Choi CS, Jee YK, Gho YS, Ryu SH, Kim YK. Gut microbe-derived extracellular vesicles induce insulin resistance, thereby impairing glucose metabolism in skeletal muscle. *Sci Rep* 2015; **5**: 15878 [PMID: 26510393 DOI: 10.1038/srep15878]
21 **Steinberg GR**, Kemp BE. AMPK in Health and Disease. *Physiol Rev* 2009; **89**: 1025-1078 [PMID: 19584320 DOI: 10.1152/physrev.00011.2008]
22 **Takagaki A**, Yoshioka Y, Yamashita Y, Nagano T, Ikeda M, Hara-Terawaki A, Seto R, Ashida H. Effects of Microbial Metabolites of (-)-Epigallocatechin Gallate on Glucose Uptake in L6 Skeletal Muscle Cell and Glucose Tolerance in ICR Mice. *Biol Pharm Bull* 2019; **42**: 212-221 [PMID: 30713253 DOI: 10.1248/bpb.b18-00612]
23 **Takagaki A**, Kato Y, Nanjo F. Isolation and characterization of rat intestinal bacteria involved in biotransformation of (-)-epigallocatechin. *Arch Microbiol* 2014; **196**: 681-695 [PMID: 24947740 DOI: 10.1007/s00203-014-1006-y]
24 **Wu H**, Tremaroli V, Schmidt C, Lundqvist A, Olsson LM, Krämer M, Gummesson A, Perkins R, Bergström G, Bäckhed F. The Gut Microbiota in Prediabetes and Diabetes: A Population-Based Cross-Sectional Study. *Cell Metab* 2020; **32**: 379-390. e3 [PMID: 32652044 DOI: 10.1016/j.cmet.2020.06.011]
25 **Thorens B**. GLUT2, glucose sensing and glucose homeostasis. *Diabetologia* 2015; **58**: 221-232 [PMID: 25421524 DOI: 10.1007/s00125-014-3451-1]
26 **Liu Y**, Dentin R, Chen D, Hedrick S, Ravnskjaer K, Schenk S, Milne J, Meyers DJ, Cole P, Yates J 3rd, Olefsky J, Guarente L, Montminy M. A fasting inducible switch modulates gluconeogenesis *via* activator/coactivator exchange. *Nature* 2008; **456**: 269-273 [PMID: 18849969 DOI: 10.1038/nature07349]
27 **Puigserver P**, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H, Accili D, Spiegelman BM. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature* 2003; **423**: 550-555 [PMID: 12754525 DOI: 10.1038/nature01667]
28 **Lee HY**, Birkenfeld AL, Jornayvaz FR, Jurczak MJ, Kanda S, Popov V, Frederick DW, Zhang D, Guigni B, Bharadwaj KG, Choi CS, Goldberg IJ, Park JH, Petersen KF, Samuel VT, Shulman GI. Apolipoprotein CIII overexpressing mice are predisposed to diet-induced hepatic steatosis and hepatic insulin resistance. *Hepatology* 2011; **54**: 1650-1660 [PMID: 21793029 DOI: 10.1002/hep.24571]
29 **Yoshida H**, Ishii M, Akagawa M. Propionate suppresses hepatic gluconeogenesis *via* GPR43/AMPK signaling pathway. *Arch Biochem Biophys* 2019; **672**: 108057 [PMID: 31356781 DOI: 10.1016/j.abb.2019.07.022]
30 **den Besten G**, Lange K, Havinga R, van Dijk TH, Gerding A, van Eunen K, Müller M, Groen AK, Hooiveld GJ, Bakker BM, Reijngoud DJ. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol* 2013; **305**: G900-G910 [PMID: 24136789 DOI: 10.1152/ajpgi.00265.2013]
31 **Boets E**, Gomand SV, Deroover L, Preston T, Vermeulen K, De Preter V, Hamer HM, Van den Mooter G, De Vuyst L, Courtin CM, Annaert P, Delcour JA, Verbeke KA. Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. *J Physiol* 2017; **595**: 541-555 [PMID: 27510655 DOI: 10.1113/JP272613]
32 **Zhang L**, Yang G, Untereiner A, Ju Y, Wu L, Wang R. Hydrogen sulfide impairs glucose utilization and increases gluconeogenesis in hepatocytes. *Endocrinology* 2013; **154**: 114-126 [PMID: 23183179 DOI: 10.1210/en.2012-1658]
33 **Chen S**, Henderson A, Petriello MC, Romano KA, Gearing M, Miao J, Schell M, Sandoval-Espinola WJ, Tao J, Sha B, Graham M, Crooke R, Kleinridders A, Balskus EP, Rey FE, Morris AJ, Biddinger SB. Trimethylamine N-Oxide Binds and Activates PERK to Promote Metabolic Dysfunction. *Cell Metab* 2019; **30**: 1141-1151. e5 [PMID: 31543404 DOI: 10.1016/j.cmet.2019.08.021]
34 **Dordević D**, Jančíková S, Vítězová M, Kushkevych I. Hydrogen sulfide toxicity in the gut environment: Meta-analysis of sulfate-reducing and lactic acid bacteria in inflammatory processes. *J Adv Res* 2021; **27**: 55-69 [PMID: 33318866 DOI: 10.1016/j.jare.2020.03.003]
35 **Jain SK**, Bull R, Rains JL, Bass PF, Levine SN, Reddy S, McVie R, Bocchini JA. Low levels of hydrogen sulfide in the blood of diabetes patients and streptozotocin-treated rats causes vascular inflammation? *Antioxid Redox Signal* 2010; **12**: 1333-1337 [PMID: 20092409 DOI: 10.1089/ars.2009.2956]
36 **Suzuki K**, Sagara M, Aoki C, Tanaka S, Aso Y. Clinical Implication of Plasma Hydrogen Sulfide Levels in Japanese Patients with Type 2 Diabetes. *Intern Med* 2017; **56**: 17-21 [PMID: 28049995 DOI: 10.2169/internalmedicine.56.7403]
37 **Rath S**, Rud T, Pieper DH, Vital M. Potential TMA-Producing Bacteria Are Ubiquitously Found in Mammalia. *Front Microbiol* 2019; **10**: 2966 [PMID: 31998260 DOI: 10.3389/fmicb.2019.02966]
38 **Tang WH**, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013; **368**: 1575-1584 [PMID: 23614584 DOI: 10.1056/NEJMoa1109400]
39 **Wang Z**, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X,

Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011; **472**: 57-63 [PMID: 21475195 DOI: 10.1038/nature09922]

40 **Svingen GF**, Schartum-Hansen H, Pedersen ER, Ueland PM, Tell GS, Mellgren G, Njølstad PR, Seifert R, Strand E, Karlsson T, Nygård O. Prospective Associations of Systemic and Urinary Choline Metabolites with Incident Type 2 Diabetes. *Clin Chem* 2016; **62**: 755-765 [PMID: 26980210 DOI: 10.1373/clinchem.2015.250761]

41 **Dambrova M**, Latkovskis G, Kuka J, Strele I, Konrade I, Grinberga S, Hartmane D, Pugovics O, Erglis A, Liepinsh E. Diabetes is Associated with Higher Trimethylamine N-oxide Plasma Levels. *Exp Clin Endocrinol Diabetes* 2016; **124**: 251-256 [PMID: 27123785 DOI: 10.1055/s-0035-1569330]

42 **Miao J**, Ling AV, Manthena PV, Gearing ME, Graham MJ, Crooke RM, Croce KJ, Esquejo RM, Clish CB; Morbid Obesity Study Group, Vicent D, Biddinger SB. Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. *Nat Commun* 2015; **6**: 6498 [PMID: 25849138 DOI: 10.1038/ncomms7498]

43 **Gao X**, Liu X, Xu J, Xue C, Xue Y, Wang Y. Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. *J Biosci Bioeng* 2014; **118**: 476-481 [PMID: 24721123 DOI: 10.1016/j.jbiosc.2014.03.001]

44 **Brown MS**, Goldstein JL. Selective *vs* total insulin resistance: a pathogenic paradox. *Cell Metab* 2008; **7**: 95-96 [PMID: 18249166 DOI: 10.1016/j.cmet.2007.12.009]

45 **Russell WR**, Duncan SH, Scobbie L, Duncan G, Cantlay L, Calder AG, Anderson SE, Flint HJ. Major phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of protein. *Mol Nutr Food Res* 2013; **57**: 523-535 [PMID: 23349065 DOI: 10.1002/mnfr.201200594]

46 **Hoyles L**, Fernández-Real JM, Federici M, Serino M, Abbott J, Charpentier J, Heymes C, Luque JL, Anthony E, Barton RH, Chilloux J, Myridakis A, Martinez-Gili L, Moreno-Navarrete JM, Benhamed F, Azalbert V, Blasco-Baque V, Puig J, Xifra G, Ricart W, Tomlinson C, Woodbridge M, Cardellini M, Davato F, Cardolini I, Porzio O, Gentileschi P, Lopez F, Foufelle F, Butcher SA, Holmes E, Nicholson JK, Postic C, Burcelin R, Dumas ME. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* 2018; **24**: 1070-1080 [PMID: 29942096 DOI: 10.1038/s41591-018-0061-3]

47 **den Besten G**, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, Oosterveer MH, Jonker JW, Groen AK, Reijngoud DJ, Bakker BM. Short-Chain Fatty Acids Protect Against High-Fat Diet-Induced Obesity *via* a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes* 2015; **64**: 2398-2408 [PMID: 25695945 DOI: 10.2337/db14-1213]

48 **Gao Z**, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009; **58**: 1509-1517 [PMID: 19366864 DOI: 10.2337/db08-1637]

49 **Mollica MP**, Mattace Raso G, Cavaliere G, Trinchese G, De Filippo C, Aceto S, Prisco M, Pirozzi C, Di Guida F, Lama A, Crispino M, Tronino D, Di Vaio P, Berni Canani R, Calignano A, Meli R. Butyrate Regulates Liver Mitochondrial Function, Efficiency, and Dynamics in Insulin-Resistant Obese Mice. *Diabetes* 2017; **66**: 1405-1418 [PMID: 28223285 DOI: 10.2337/db16-0924]

50 **Sato S**, Jung H, Nakagawa T, Pawlosky R, Takeshima T, Lee WR, Sakiyama H, Laxman S, Wynn RM, Tu BP, MacMillan JB, De Brabander JK, Veech RL, Uyeda K. Metabolite Regulation of Nuclear Localization of Carbohydrate-response Element-binding Protein (ChREBP): ROLE OF AMP AS AN ALLOSTERIC INHIBITOR. *J Biol Chem* 2016; **291**: 10515-10527 [PMID: 26984404 DOI: 10.1074/jbc.M115.708982]

51 **Yamashita H**, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M, Hiemori M, Tsuji H. Improvement of obesity and glucose tolerance by acetate in Type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* 2007; **71**: 1236-1243 [PMID: 17485860 DOI: 10.1271/bbb.60668]

52 **Kondo T**, Kishi M, Fushimi T, Kaga T. Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation. *J Agric Food Chem* 2009; **57**: 5982-5986 [PMID: 19469536 DOI: 10.1021/jf900470c]

53 **Sahuri-Arisoylu M**, Brody LP, Parkinson JR, Parkes H, Navaratnam N, Miller AD, Thomas EL, Frost G, Bell JD. Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *Int J Obes (Lond)* 2016; **40**: 955-963 [PMID: 26975441 DOI: 10.1038/ijo.2016.23]

54 **Kahn BB**, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; **106**: 473-481 [PMID: 10953022 DOI: 10.1172/jci10842]

55 **Goto T**, Kim YI, Furuzono T, Takahashi N, Yamakuni K, Yang HE, Li Y, Ohue R, Nomura W, Sugawara T, Yu R, Kitamura N, Park SB, Kishino S, Ogawa J, Kawada T. 10-oxo-12(Z)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, potently activates PPARγ and stimulates adipogenesis. *Biochem Biophys Res Commun* 2015; **459**: 597-603 [PMID: 25749343 DOI: 10.1016/j.bbrc.2015.02.154]

56 **Guilherme A**, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 2008; **9**: 367-377 [PMID: 18401346 DOI: 10.1038/nrm2391]

57 **Perry RJ**, Camporez JG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, Jurczak MJ, Abudukadier

A, Han MS, Zhang XM, Ruan HB, Yang X, Caprio S, Kaech SM, Sul HS, Birnbaum MJ, Davis RJ, Cline GW, Petersen KF, Shulman GI. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. *Cell* 2015; **160**: 745-758 [PMID: 25662011 DOI: 10.1016/j.cell.2015.01.012]

58 **Morigny P**, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. *Biochimie* 2016; **125**: 259-266 [PMID: 26542285 DOI: 10.1016/j.biochi.2015.10.024]

59 **Hong YH**, Nishimura Y, Hishikawa D, Tsuzuki H, Miyahara H, Gotoh C, Choi KC, Feng DD, Chen C, Lee HG, Katoh K, Roh SG, Sasaki S. Acetate and propionate short chain fatty acids stimulate adipogenesis *via* GPCR43. *Endocrinology* 2005; **146**: 5092-5099 [PMID: 16123168 DOI: 10.1210/en.2005-0545]

60 **Ge H**, Li X, Weiszmann J, Wang P, Baribault H, Chen JL, Tian H, Li Y. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology* 2008; **149**: 4519-4526 [PMID: 18499755 DOI: 10.1210/en.2008-0059]

61 **Jocken JWE**, González Hernández MA, Hoebers NTH, van der Beek CM, Essers YPG, Blaak EE, Canfora EE. Short-Chain Fatty Acids Differentially Affect Intracellular Lipolysis in a Human White Adipocyte Model. *Front Endocrinol (Lausanne)* 2017; **8**: 372 [PMID: 29375478 DOI: 10.3389/fendo.2017.00372]

62 **Scheja L**, Heeren J. The endocrine function of adipose tissues in health and cardiometabolic disease. *Nat Rev Endocrinol* 2019; **15**: 507-524 [PMID: 31296970 DOI: 10.1038/s41574-019-0230-6]

63 **Smith U**, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. *J Intern Med* 2016; **280**: 465-475 [PMID: 27699898 DOI: 10.1111/joim.12540]

64 **Zatterale F**, Longo M, Naderi J, Raciti GA, Desiderio A, Miele C, Beguinot F. Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Front Physiol* 2019; **10**: 1607 [PMID: 32063863 DOI: 10.3389/fphys.2019.01607]

65 **Shoelson SE**, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; **116**: 1793-1801 [PMID: 16823477 DOI: 10.1172/jci29069]

66 **Al-Lahham S**, Roelofsen H, Rezaee F, Weening D, Hoek A, Vonk R, Venema K. Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *Eur J Clin Invest* 2012; **42**: 357-364 [PMID: 21913915 DOI: 10.1111/j.1365-2362.2011.02590.x]

67 **Ohira H**, Fujioka Y, Katagiri C, Mamoto R, Aoyama-Ishikawa M, Amako K, Izumi Y, Nishiumi S, Yoshida M, Usami M, Ikeda M. Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages. *J Atheroscler Thromb* 2013; **20**: 425-442 [PMID: 23470566 DOI: 10.5551/jat.15065]

68 **Townsend KL**, Tseng YH. Brown fat fuel utilization and thermogenesis. *Trends Endocrinol Metab* 2014; **25**: 168-177 [PMID: 24389130 DOI: 10.1016/j.tem.2013.12.004]

69 **Lizcano F**. The Beige Adipocyte as a Therapy for Metabolic Diseases. *Int J Mol Sci* 2019; **20** [PMID: 31614705 DOI: 10.3390/ijms20205058]

70 **O'Callaghan A**, van Sinderen D. Bifidobacteria and Their Role as Members of the Human Gut Microbiota. *Front Microbiol* 2016; **7**: 925 [PMID: 27379055 DOI: 10.3389/fmicb.2016.00925]

71 **Shimizu J**, Kubota T, Takada E, Takai K, Fujiwara N, Arimitsu N, Murayama MA, Ueda Y, Wakisaka S, Suzuki T, Suzuki N. Propionate-producing bacteria in the intestine may associate with skewed responses of IL10-producing regulatory T cells in patients with relapsing polychondritis. *PLoS One* 2018; **13**: e0203657 [PMID: 30235279 DOI: 10.1371/journal.pone.0203657]

72 **Lordan C**, Thapa D, Ross RP, Cotter PD. Potential for enriching next-generation health-promoting gut bacteria through prebiotics and other dietary components. *Gut Microbes* 2020; **11**: 1-20 [PMID: 31116628 DOI: 10.1080/19490976.2019.1613124]

73 **Hanatani S**, Motoshima H, Takaki Y, Kawasaki S, Igata M, Matsumura T, Kondo T, Senokuchi T, Ishii N, Kawashima J, Kukidome D, Shimoda S, Nishikawa T, Araki E. Acetate alters expression of genes involved in beige adipogenesis in 3T3-L1 cells and obese KK-Ay mice. *J Clin Biochem Nutr* 2016; **59**: 207-214 [PMID: 27895388 DOI: 10.3164/jcbn.16-23]

74 **Li Z**, Yi CX, Katiraei S, Kooijman S, Zhou E, Chung CK, Gao Y, van den Heuvel JK, Meijer OC, Berbée JFP, Heijink M, Giera M, Willems van Dijk K, Groen AK, Rensen PCN, Wang Y. Butyrate reduces appetite and activates brown adipose tissue *via* the gut-brain neural circuit. *Gut* 2018; **67**: 1269-1279 [PMID: 29101261 DOI: 10.1136/gutjnl-2017-314050]

75 **Yamashita H**, Maruta H, Jozuka M, Kimura R, Iwabuchi H, Yamato M, Saito T, Fujisawa K, Takahashi Y, Kimoto M, Hiemori M, Tsuji H. Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* 2009; **73**: 570-576 [PMID: 19270372 DOI: 10.1271/bbb.80634]

76 **Bouter K**, Bakker GJ, Levin E, Hartstra AV, Kootte RS, Udayappan SD, Katiraei S, Bahler L, Gilijamse PW, Tremaroli V, Stahlman M, Holleman F, van Riel NAW, Verberne HJ, Romijn JA, Dallinga-Thie GM, Serlie MJ, Ackermans MT, Kemper EM, Willems van Dijk K, Backhed F, Groen AK, Nieuwdorp M. Differential metabolic effects of oral butyrate treatment in lean *vs* metabolic syndrome subjects. *Clin Transl Gastroenterol* 2018; **9**: 155 [PMID: 29799027 DOI: 10.1038/s41424-018-0025-4]

77 **Canfora EE**, van der Beek CM, Jocken JWE, Goossens GH, Holst JJ, Olde Damink SWM, Lenaerts K, Dejong CHC, Blaak EE. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci Rep* 2017; **7**: 2360 [PMID: 28539646 DOI: 10.1038/s41598-017-02546-x]

78 **van der Beek CM**, Canfora EE, Lenaerts K, Troost FJ, Damink SWMO, Holst JJ, Masclee AAM, Dejong CHC, Blaak EE. Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clin Sci (Lond)* 2016; **130**: 2073-2082 [PMID: 27439969 DOI: 10.1042/CS20160263]

79 **Chambers ES**, Byrne CS, Aspey K, Chen Y, Khan S, Morrison DJ, Frost G. Acute oral sodium propionate supplementation raises resting energy expenditure and lipid oxidation in fasted humans. *Diabetes Obes Metab* 2018; **20**: 1034-1039 [PMID: 29134744 DOI: 10.1111/dom.13159]

80 **Christie S**, Wittert GA, Li H, Page AJ. Involvement of TRPV1 Channels in Energy Homeostasis. *Front Endocrinol (Lausanne)* 2018; **9**: 420 [PMID: 30108548 DOI: 10.3389/fendo.2018.00420]

81 **Kim M**, Furuzono T, Yamakuni K, Li Y, Kim YI, Takahashi H, Ohue-Kitano R, Jheng HF, Takahashi N, Kano Y, Yu R, Kishino S, Ogawa J, Uchida K, Yamazaki J, Tominaga M, Kawada T, Goto T. 10-oxo-12(*Z*)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, enhances energy metabolism by activation of TRPV1. *FASEB J* 2017; **31**: 5036-5048 [PMID: 28754711 DOI: 10.1096/fj.201700151R]

82 **Takahashi Y**, Kushiro M, Shinohara K, Ide T. Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice. *Comp Biochem Physiol B Biochem Mol Biol* 2002; **133**: 395-404 [PMID: 12431407 DOI: 10.1016/s1096-4959(02)00164-1]

83 **Park Y**, Park Y. Conjugated fatty acids increase energy expenditure in part by increasing voluntary movement in mice. *Food Chem* 2012; **133**: 400-409 [PMID: 25683412 DOI: 10.1016/j.foodchem.2012.01.051]

84 **Lee HY**, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y. Human originated bacteria, Lactobacillus rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochim Biophys Acta* 2006; **1761**: 736-744 [PMID: 16807088 DOI: 10.1016/j.bbalip.2006.05.007]

85 **Koepsell H**. Glucose transporters in the small intestine in health and disease. *Pflugers Arch* 2020; **472**: 1207-1248 [PMID: 32829466 DOI: 10.1007/s00424-020-02439-5]

86 **Pavlic M**, Xiao C, Szeto L, Patterson BW, Lewis GF. Insulin acutely inhibits intestinal lipoprotein secretion in humans in part by suppressing plasma free fatty acids. *Diabetes* 2010; **59**: 580-587 [PMID: 20028946 DOI: 10.2337/db09-1297]

87 **Ussar S**, Haering MF, Fujisaka S, Lutter D, Lee KY, Li N, Gerber GK, Bry L, Kahn CR. Regulation of Glucose Uptake and Enteroendocrine Function by the Intestinal Epithelial Insulin Receptor. *Diabetes* 2017; **66**: 886-896 [PMID: 28096258 DOI: 10.2337/db15-1349]

88 **Federico LM**, Naples M, Taylor D, Adeli K. Intestinal insulin resistance and aberrant production of apolipoprotein B48 Lipoproteins in an animal model of insulin resistance and metabolic dyslipidemia: evidence for activation of protein tyrosine phosphatase-1B, extracellular signal-related kinase, and sterol regulatory element-binding protein-1c in the fructose-fed hamster intestine. *Diabetes* 2006; **55**: 1316-1326 [PMID: 16644688 DOI: 10.2337/db04-1084]

89 **Croset M**, Rajas F, Zitoun C, Hurot JM, Montano S, Mithieux G. Rat small intestine is an insulin-sensitive gluconeogenic organ. *Diabetes* 2001; **50**: 740-746 [PMID: 11289037 DOI: 10.2337/diabetes.50.4.740]

90 **Rajas F**, Croset M, Zitoun C, Montano S, Mithieux G. Induction of PEPCK gene expression in insulinopenia in rat small intestine. *Diabetes* 2000; **49**: 1165-1168 [PMID: 10909974 DOI: 10.2337/diabetes.49.7.1165]

91 **De Vadder F**, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. Microbiota-generated metabolites promote metabolic benefits *via* gut-brain neural circuits. *Cell* 2014; **156**: 84-96 [PMID: 24412651 DOI: 10.1016/j.cell.2013.12.016]

92 **De Vadder F**, Kovatcheva-Datchary P, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. Microbiota-Produced Succinate Improves Glucose Homeostasis *via* Intestinal Gluconeogenesis. *Cell Metab* 2016; **24**: 151-157 [PMID: 27411015 DOI: 10.1016/j.cmet.2016.06.013]

93 **Troy S**, Soty M, Ribeiro L, Laval L, Migrenne S, Fioramonti X, Pillot B, Fauveau V, Aubert R, Viollet B, Foretz M, Leclerc J, Duchampt A, Zitoun C, Thorens B, Magnan C, Mithieux G, Andreelli F. Intestinal gluconeogenesis is a key factor for early metabolic changes after gastric bypass but not after gastric lap-band in mice. *Cell Metab* 2008; **8**: 201-211 [PMID: 18762021 DOI: 10.1016/j.cmet.2008.08.008]

94 **Duraffourd C**, De Vadder F, Goncalves D, Delaere F, Penhoat A, Brusset B, Rajas F, Chassard D, Duchampt A, Stefanutti A, Gautier-Stein A, Mithieux G. Mu-opioid receptors and dietary protein stimulate a gut-brain neural circuitry limiting food intake. *Cell* 2012; **150**: 377-388 [PMID: 22771138 DOI: 10.1016/j.cell.2012.05.039]

95 **De Vadder F**, Plessier F, Gautier-Stein A, Mithieux G. Vasoactive intestinal peptide is a local mediator in a gut-brain neural axis activating intestinal gluconeogenesis. *Neurogastroenterol Motil* 2015; **27**: 443-448 [PMID: 25586379 DOI: 10.1111/nmo.12508]

96 **Mithieux G**, Misery P, Magnan C, Pillot B, Gautier-Stein A, Bernard C, Rajas F, Zitoun C. Portal sensing of intestinal gluconeogenesis is a mechanistic link in the diminution of food intake induced by diet protein. *Cell Metab* 2005; **2**: 321-329 [PMID: 16271532 DOI: 10.1016/j.cmet.2005.09.010]

97 **Soty M**, Gautier-Stein A, Rajas F, Mithieux G. Gut-Brain Glucose Signaling in Energy Homeostasis. *Cell Metab* 2017; **25**: 1231-1242 [PMID: 28591631 DOI: 10.1016/j.cmet.2017.04.032]

98 **Yan Y**, Zhou Z, Kong F, Feng S, Li X, Sha Y, Zhang G, Liu H, Zhang H, Wang S, Hu C, Zhang X. Roux-en-Y Gastric Bypass Surgery Suppresses Hepatic Gluconeogenesis and Increases Intestinal

Gluconeogenesis in a T2DM Rat Model. *Obes Surg* 2016; **26**: 2683-2690 [PMID: 27038047 DOI: 10.1007/s11695-016-2157-5]

99 **Zhang X**, Yang S, Chen J, Su Z. Unraveling the Regulation of Hepatic Gluconeogenesis. *Front Endocrinol (Lausanne)* 2018; **9**: 802 [PMID: 30733709 DOI: 10.3389/fendo.2018.00802]

100 **Kovatcheva-Datchary P**, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, Hallen A, Martens E, Björck I, Bäckhed F. Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella. *Cell Metab* 2015; **22**: 971-982 [PMID: 26552345 DOI: 10.1016/j.cmet.2015.10.001]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 745-766

DOI: 10.4239/wjd.v12.i6.745

ISSN 1948-9358 (online)

REVIEW

Alzheimer's disease and type 2 diabetes mellitus: Pathophysiologic and pharmacotherapeutics links

Milagros Rojas, Mervin Chávez-Castillo, Jordan Bautista, Ángel Ortega, Manuel Nava, Juan Salazar, Edgar Díaz-Camargo, Oscar Medina, Joselyn Rojas-Quintero, Valmore Bermúdez

ORCID number: Milagros Rojas 0000-0003-2764-8846; Mervin Chávez-Castillo 0000-0001-8511-0230; Jordan Bautista 0000-0003-4059-7086; Ángel Ortega 0000-0002-7180-4765; Manuel Nava 0000-0001-9769-1693; Juan Salazar 0000-0003-4211-528X; Edgar Díaz-Camargo 0000-0002-7349-3059; Oscar Medina 0000-0002-2472-1238; Joselyn Rojas-Quintero 0000-0003-4994-075X; Valmore Bermúdez 0000-0003-1880-8887.

Author contributions: Rojas M, Chávez-Castillo M and Bautista J conceived of the idea of the study and determined its general focus; Rojas M, Chávez-Castillo M, Bautista J, Ortega Á and Nava M contributed to the literature and wrote and revised the manuscript; Rojas M, Chávez-Castillo M, Salazar J, Bermúdez V, Díaz-Camargo E, Medina O and Rojas-Quintero J contributed to the review study and corrected the manuscript; all authors reviewed and approved the final version of the manuscript.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Milagros Rojas, Mervin Chávez-Castillo, Jordan Bautista, Ángel Ortega, Manuel Nava, Juan Salazar, Endocrine and Metabolic Diseases Research Center, School of Medicine, University of Zulia, Maracaibo 4004, Venezuela

Edgar Díaz-Camargo, Oscar Medina, Universidad Simón Bolívar, Facultad de Ciencias Jurídicas y Sociales, Cúcuta 540006, Colombia

Joselyn Rojas-Quintero, Pulmonary and Critical Care Medicine Department, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02155, United States

Valmore Bermúdez, Universidad Simón Bolívar, Facultad de Ciencias de la Salud, Barranquilla 080001, Colombia

Corresponding author: Juan Salazar, MD, Doctor, Endocrine and Metabolic Diseases Research Center, School of Medicine, University of Zulia, 20th Avenue, Maracaibo 4004, Venezuela. juanjsv18@hotmail.com

Abstract

At present, Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are two highly prevalent disorders worldwide, especially among elderly individuals. T2DM appears to be associated with cognitive dysfunction, with a higher risk of developing neurocognitive disorders, including AD. These diseases have been observed to share various pathophysiological mechanisms, including alterations in insulin signaling, defects in glucose transporters (GLUTs), and mitochondrial dysfunctions in the brain. Therefore, the aim of this review is to summarize the current knowledge regarding the molecular mechanisms implicated in the association of these pathologies as well as recent therapeutic alternatives. In this context, the hyperphosphorylation of tau and the formation of neurofibrillary tangles have been associated with the dysfunction of the phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways in the nervous tissues as well as the decrease in the expression of GLUT-1 and GLUT-3 in the different areas of the brain, increase in reactive oxygen species, and production of mitochondrial alterations that occur in T2DM. These findings have contributed to the implementation of overlapping pharmacological interventions based on the use of insulin and antidiabetic drugs, or, more recently, azeliragon, amylin, among others, which have shown possible beneficial effects in diabetic patients diagnosed with AD.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Venezuela

Peer-review report's scientific quality classification

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

Received: February 13, 2021
Peer-review started: February 13, 2021
First decision: March 16, 2021
Revised: March 20, 2021
Accepted: May 21, 2021
Article in press: May 21, 2021
Published online: June 15, 2021

P-Reviewer: Ugo O
S-Editor: Gao CC
L-Editor: A
P-Editor: Ma YJ

Key Words: Alzheimer's disease; Type 2 diabetes mellitus; Oxidative stress; Islet amyloid polypeptide; Glucagon-like peptide 1; Cognitive dysfunction; Hypoglycemic agents

Core Tip: Alzheimer's disease and type 2 diabetes mellitus are highly prevalent chronic diseases that have shown a significant association. Important pathways have shown to be involved in this relationship, including the phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways, among others. This has led to the development of therapeutic approaches that can overlap and address both diseases. Some of the most promising interventions include the use of azeliragon, amylin, and glucagon-like peptide-1.

Citation: Rojas M, Chávez-Castillo M, Bautista J, Ortega Á, Nava M, Salazar J, Díaz-Camargo E, Medina O, Rojas-Quintero J, Bermúdez V. Alzheimer's disease and type 2 diabetes mellitus: Pathophysiologic and pharmacotherapeutics links. *World J Diabetes* 2021; 12(6): 745-766
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/745.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.745

INTRODUCTION

Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are highly prevalent diseases in elderly patients[1,2]. According to the International Diabetes Federation, 1 in 11 people had DM in 2019, amounting to approximately 463 million cases worldwide, of which 90% is attributed to T2DM. These alarming numbers have quadrupled since 1990, with a prevalence of 9.3% in adults[3-5]. Moreover, numerous complications have been associated with this disorder, among which are renal disease [6], retinopathy[7], dermopathy[8], peripheral vasculopathy[9], and cognitive alterations[10]. The latter have been investigated recently, due to the prominent link between T2DM and any type of neurocognitive disorder, especially AD.

As a progressive, degenerative, and irreversible disorder, AD is characterized by neuronal loss, the formation of senile plaques composed of extracellular deposits of amyloid beta, and the presence of neurofibrillary tangles (NT) in neuronal microtubules [11]. Recent studies have demonstrated a higher incidence of AD in patients with T2DM in comparison to those without comorbidities. This epidemiologic association has spawned various hypothetical pathophysiological links between both diseases[12]. These studies focus on the role of insulin as a necessary hormone for glucose metabolism in the central nervous system (CNS), with receptors widely localized in the hippocampus, frontal area, and entorhinal cortex, which are all structures involved in memory and learning[13].

Insulin resistance (IR) is a key alteration of this hormone's signaling and is the pathogenic cornerstone of T2DM, where it promotes a chronic state of hyperinsulinemia and hyperglycemia[14]. IR has been associated with the disruption of amyloid protein metabolism in the brain, tau phosphorylation in microtubules[15], and the formation of reactive oxygen species (ROS) in the mitochondria. Thus, these conditions may contribute to the pathogenesis and development of AD.

Therefore, the aim of this narrative review is to summarize the current knowledge on the molecular mechanisms linking T2DM with the development of AD, along with possible emerging therapeutic strategies.

MOLECULAR BASIS OF GLUCOSE METABOLISM IN THE BRAIN

Of all organs, the brain requires the largest energetic demand[16] (Figure 1). Under physiological conditions, a constant supply of glucose, which is the main substrate, is necessary for a normal metabolic functionality. Glucose traffic to the brain is regulated in the endothelial wall of the capillaries by the blood-brain barrier (BBB), a structure composed of two semipermeable membranes, one luminal and one abluminal[17].

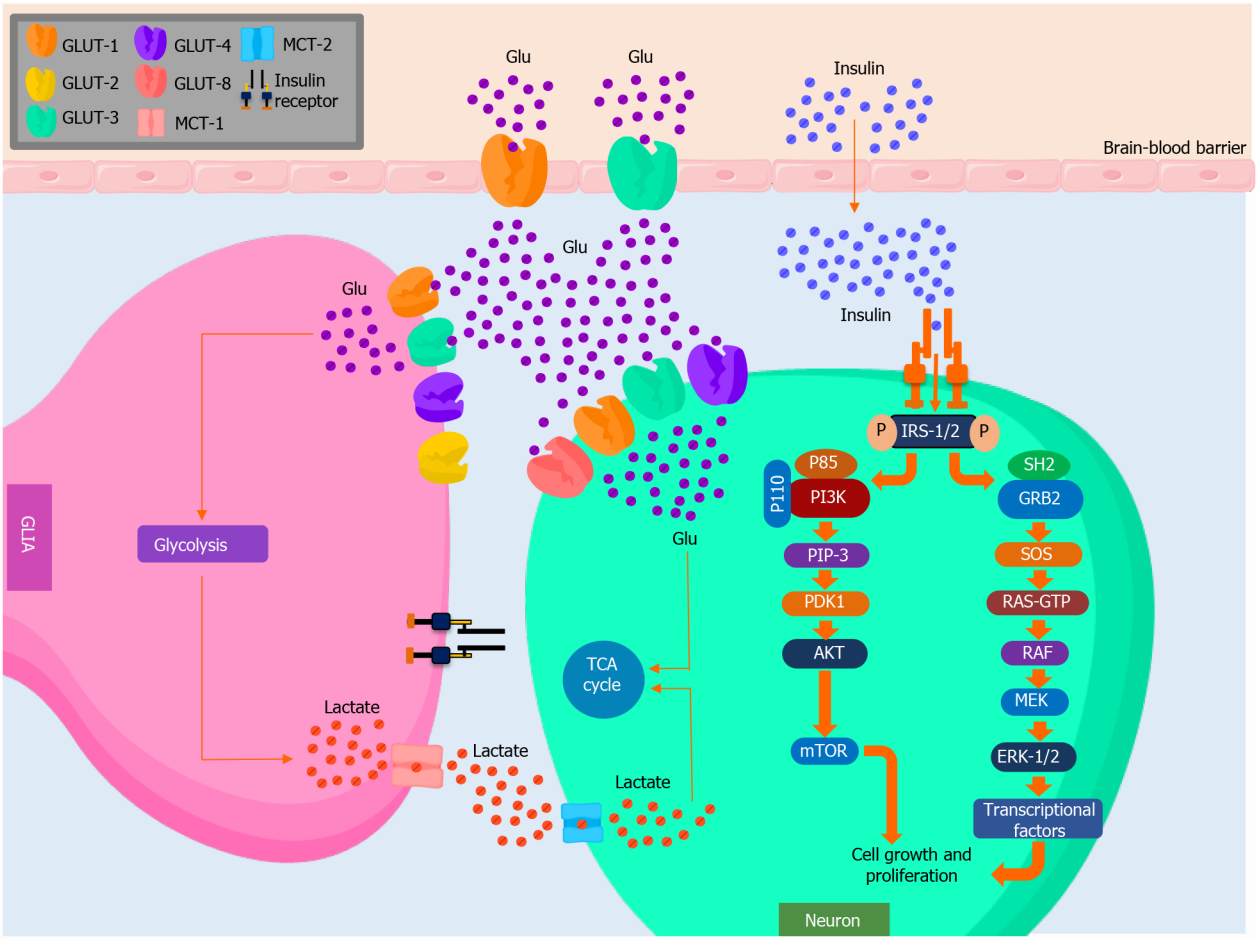

Figure 1 Molecular basis of brain glucose and insulin metabolism. Glucose transporter (GLUT)-1 and GLUT-3 are widely expressed in the brain blood barrier (BBB), allowing glucose transport. Likewise, GLUT-1, GLUT-2, GLUT-3, and GLUT-4 are present in the membrane of glial cells, whereas GLUT-1, GLUT-3, GLUT-4, and GLUT-8 are expressed in neurons. These allow the transport of glucose to the intracellular environment, ready for energetic metabolism. On the other hand, it has been proposed that insulin crosses the BBB through a saturable transporter and then couples to the insulin receptor substrate (IRS) in the neuronal membrane, causing a conformational change that phosphorylates IRS-1/2, which mainly activates the AKT and extracellular signal-regulated kinase (ERK)-1/2 pathways. After a phosphorylation cascade, this activates mTOR and various transcriptional factors involved in the growth and cellular differentiation of the nervous system. Glu: Glucose; GLUT: Glucose transporters; IR: Insulin receptor; MCT: Monocarboxylate transporter; IRS: Insulin receptor substrate; GRB2: Growth factor receptor-bound protein 2; SOS: Son of Sevenless homolog; RAF: Rapidly accelerated fibrosarcoma kinase; MEK: Mitogen-activated protein kinases; ERK: Extracellular signal-regulated kinase; PI3K: Phosphatidylinositol 3-kinase; PIP-3: Phosphatidylinositol (3,4,5)-trisphosphate; PDK1: Phosphoinositide-dependent kinase-1; AKT: Protein kinase B; TCA cycle: Tricarboxylic acid cycle.

Both are aligned next to endothelial cells, regulating the transportation of hydrophilic metabolic substrates, such as carbohydrates and amino acids, in the CNS[18].

Glucose is a polar and hydrophilic molecule, preventing its diffusion through the blood capillaries. Thus, it needs a system of specific transporters[16]. Glucose transporters (GLUTs) in the cells of superior organisms are classified into two large families: the family of facilitated diffusion GLUTs and the family of sodium and glucose co-transporters. GLUTs are glycoproteins located in the plasmatic membrane. They have a range between 45 and 55 kDa and their N- and C-terminal ends are both located in the cytoplasm[19].

GLUTs in the nervous system: GLUT-1 and GLUT-3

GLUT-1 and GLUT-3 isoforms display the highest affinity for glucose. Therefore, their presence in tissues that exclusively depend on glucose for their metabolic requirements is extremely important[16]. They are expressed in the endothelial cells of the BBB as well as the plasma membranes of neurons and glial cells[20].

The GLUT-1 gene is located in the 1p35.31.3 chromosome, which is widely expressed in numerous tissues, and is considered the main GLUT of the BBB[16]. It is 3-4 times more abundant in the luminal membrane than the abluminal membrane and mediates the transport of glucose from the blood to astrocytes. GLUT-1 isoforms vary on molecular weight. 55-kD GLUT-1 is expressed in the endothelial cells of the brain blood vessels, whereas 45-kD GLUT-1 is mainly expressed in astrocytes. GLUT-1 is

highly hydrophobic, and its high glucose affinity (Km = 1-2 mmol/L) allows it to transport glucose into cells at virtually any concentration[17], with selectivity for D-glucose. Therefore, it is thought to act as a basal GLUT that maintains stable glucose levels in the CNS[20].

GLUT-3 works in harmony with GLUT-1, allowing the vectorial transport of glucose to neurons[16]. It is a high-affinity GLUT (Km = 1-2 mmol/L) found primarily in the brain, although low levels of GLUT-3 have been detected in the myocardium, placenta, liver, and muscle. Regarding its kinetic properties, it appears to alternate between the intake and release of glucose[21]. Current hypotheses suggest the facilitated transport of glucose involves conformational changes in the tertiary structure of the transporter. This is triggered by the presence of glucose in its binding site on the extracellular portion of the transporter and its progressive movement to the intracellular portion of it, where another binding site is found[19].

The expression of GLUT in the nervous system varies across different cells. Astrocytes express different isoforms of the GLUT family, including GLUT-1, GLUT-2, and GLUT-4. These cells cover 99% of the BBB and it is through these GLUT isoforms that glucose is able to cross this barrier[18]. Meanwhile, neurons express GLUT-3, GLUT-4, and GLUT-8. Despite the expression of these GLUT isoforms, it has been investigated whether astrocytes, which take glucose and release lactate, are a necessary mediator between glucose and neurons or, as the conventional hypothesis proposes, neurons receive glucose directly from the interstitial fluid of the brain in aerobic conditions[21]. Through this process, glucose would enter the glycolytic pathway and the tricarboxylic acid pathway for its later oxidation, which provides the cell with the necessary energy to maintain cellular function[17]. The aforementioned glucose transportation mechanism can become the limiting step in certain situations, including the development of hypoglycemia and other conditions, such as AD, mainly limiting blood flow, BBB permeability, and changes in GLUT-1 expression[18].

Role of insulin in the brain

Insulin is a peptide hormone necessary for maintaining glucose homeostasis[22]. In the brain, it has important neuroprotective and neuromodulatory functions, such as regulating its growth, repairing dendritic cells and neurons, and having anorexigenic effects on the hypothalamus, among other effects[23]. It is chiefly synthesized in the pancreas[24], although the presence of insulin mRNA in neurons suggests that it may be locally produced in the nervous tissues, especially in the hypothalamus, cerebral cortex, olfactory bulb, substantia nigra, and pituitary gland[16]. Insulin may also intervene in learning and memory brain functions, especially verbal memory, as evidence shows this hormone modulates the secretion of neurotransmitters, such as acetylcholine, and favors synaptic plasticity[22].

Once in the plasma, insulin can cross the BBB *via* active transportation mediated by its receptor, which is abundantly expressed in neurons and, in lower quantities, in glial cells[22]. After binding to its receptor, insulin promotes the autophosphorylation of tyrosine residues, triggering its intrinsic tyrosine kinase activity and phosphorylating the insulin receptor substrate (IRS) coupling protein in the tyrosine residue[24]. The majority of this response is coupled to IRS-1 and IRS-2, which are ubiquitously expressed and the main mediators of insulin-dependent mitogenesis, and the regulation of glucose metabolism in the majority of cell types[21]. Historically, IRS-1 was the first insulin substrate to be identified and represents the prototype of IRS family proteins, whereas IRS-2 is mainly involved in the regulation of brain growth. The phosphorylation of tyrosine residues on IRS activates Akt, which phosphorylates substrates, such as the mammalian target of rapamycin (mTOR) and the glycogen synthase kinase-3 (GSK3), among other targets[25]. Insulin also activates the extracellular signal receptor kinase (ERK) pathway by activating type 1 and type 2 ERKs[24]. These molecules can modify the expression of certain genes (c-fos, Elk-1) involved in cell growth and differentiation[22].

PATHOGENESIS OF AD: NEUROBIOLOGICAL PRINCIPLES

AD is a neurogenerative disorder that results in a gradual and irreversible deterioration of memory and other cognitive functions. It can also be frequently accompanied by other manifestations, such as psychosis, depression, and behavioral alterations[26]. Various environmental, genetic, and biologic factors participate in its pathogenesis[27,28]. Genetic data suggest that AD may be the result of the dysfunction in the amyloid protein precursor pathway, where the production of presenilin 1

(PSEN1) and presenilin 2 (PSEN2) takes place. The *PSEN1* gene is located in chromosome 14, whereas the *PSEN2* gene is located in chromosome 1[29]. Although mutations in these genes lead to the familiar autosomal presentations of AD, the presence of the E4 allele of the E apolipoprotein (APOE) is the main genetic susceptibility factor that has been identified. People with E4/4 homozygotes are eight times more likely to suffer AD compared to those who do not have these alleles[30].

Amyloid metabolism: Senile plaques

Physiologically, neuronal cells release soluble amyloid beta, which is a peptide with a molecular weight of 4 kD and a length of 42-43 amino acids. The main types of amyloid (Aβ40 and Aβ42) emerge as a product of the normal secretion of the transmembrane amyloid protein precursor after a proteolytic process that requires the participation of secretases (α β γ). α-Secretase acts on the amyloid beta peptide, promoting its breakdown in two segments, which are nexin II and soluble amyloid beta peptide, which has 16 amino acids[31].

Afterward, the α-2-macroglobulin acts forming the BA-A2M complex, which will couple to a protease enzyme to reenter the neuron[32]. During this process, the secretases cleave the BA peptide from 40 to 42 amino acids. Such enzymes include the beta secretase (acting on amino acid 1) and gamma secretase (40-42 activity). The accumulation in the interstitial tissue of insoluble 1-42 beta amyloid fragments goes through various transformations in relation to its protein structure until it acquires a folded shape that is difficult to break down. Furthermore, other stable proteins are associated with this process, such as the serum amyloid P component[33]. The presence of these structures leads to the activation of the immune system, especially the phagocytic cells of the CNS (microglia), which perpetuate the lesion due to pseudoinflammation and the release of ROS. However, recent studies have suggested that the participation of amyloid beta is attributed to its deposit on the brain blood vessels, which leads to degeneration and hemorrhages, which are important events in the physiopathology of AD[34].

Neurofibrillary metabolism: NT

Tau is a protein that is highly associated with neuronal microtubules[35]. Through its isoforms and phosphorylation, tau protein interacts with tubulin to stabilize the structure of neuronal microtubules, allowing for an efficient synaptic activity[36]. The tau hypothesis indicates that an excessive or abnormal phosphorylation of this protein results in the transformation to a paired helical filament conformation (PHF-tau). This leads to its precipitation and autoaggregation, which slows the axonal transport and causes neurodegeneration due to possible apoptosis[37]. NT can be intracellular and extracellular. Intracellular NT are hyperphosphorylated and usually found in abundance in the neuritic component of the neuritic plaque. Meanwhile, extracellular NT are the result of neuronal death and the denomination of the insoluble fibrillar skeleton and is characterized by insoluble neurofibrillary components that are difficult to proteolyze. These persist even after neuronal death as remains in the extracellular medium[38].

AD, IR, AND T2DM: PHYSIOPATHOLOGICAL LINKS

General glucose metabolism involves various intracellular processes, including glycolysis, the Krebs cycle, and oxidative phosphorylation. Likewise, it requires extracellular factors, such as its transportation from the circulation to the intracellular environment, in which insulin has a key regulating role[12]. T2DM is associated with the progressive loss of sensitivity to this hormone in a growing IR state, which is also present in AD. This outlines a possible overlap in the pathogenesis of both condi-tions [39].

T2DM has been associated with changes in cognition and cognitive dysfunction, reporting a higher risk of developing any type of neurocognitive disorder, including AD[40]. Indeed, various clinical and preclinical studies suggest that these disorders may share multiple biochemical characteristics and signaling pathways[41] (Figure 2).

Different studies have demonstrated the association between IR and AD, even in the absence of hyperglycemia or DM. In this sense, it has been found that neurocognitive functions dependent on insulin in patients with sporadic AD could play an important role in the physiopathology of this disease, causing disruptions of insulin signaling in the brains of these individuals[42]. Similarly, a second study examining AD patients non-homozygous for the APOE-ε4 allele reported the presence of fasting insulinemia

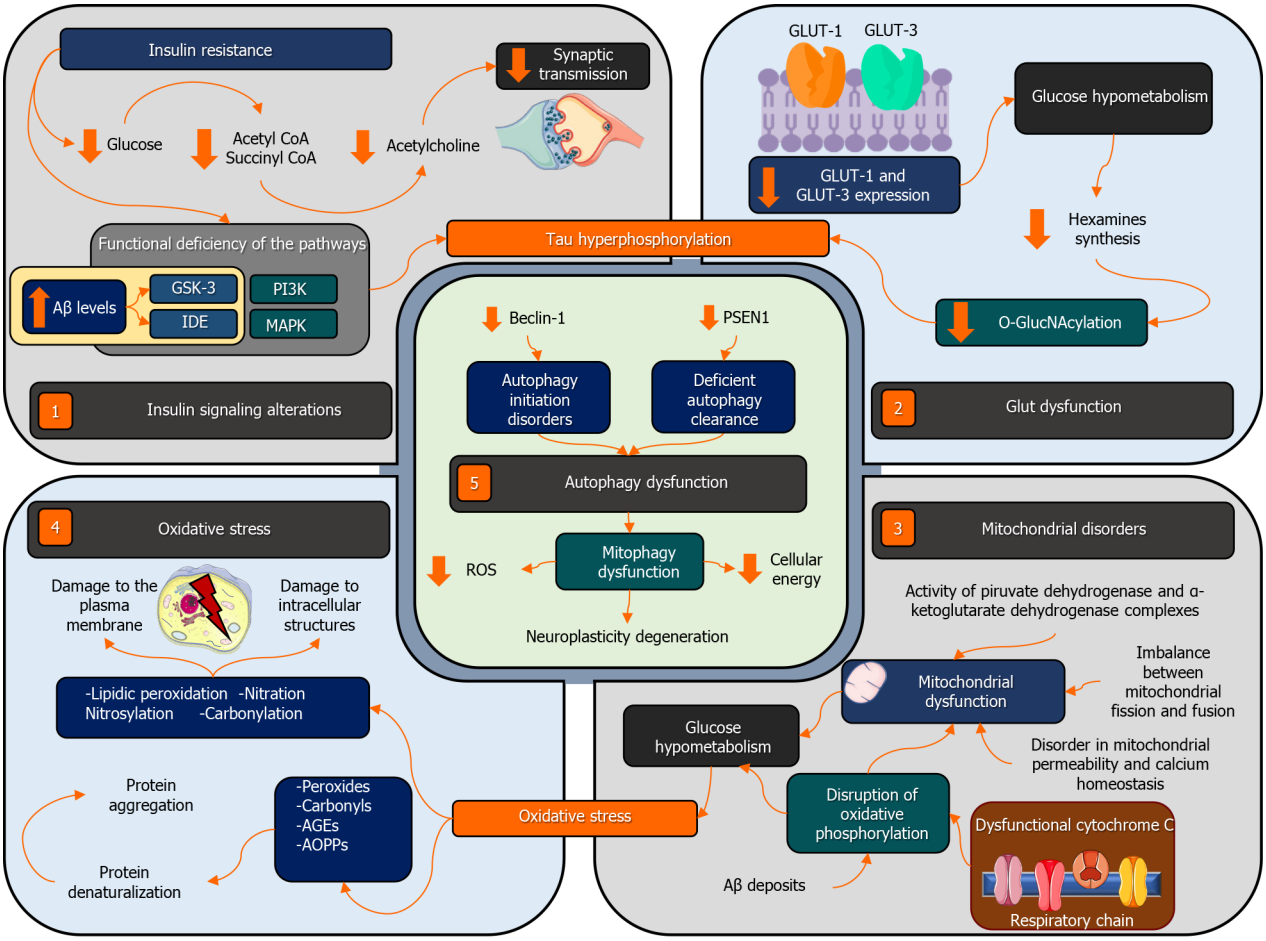

Figure 2 Pathophysiological links between Alzheimer's disease and type 2 diabetes mellitus. Alterations in insulin signaling (1), particularly insulin resistance (IR), decrease the bioavailability of intracellular glucose, altering the synthesis of acetylcholine precursors, which affects synaptic transmissions related to cognition. Likewise, IR alters the intracellular signaling cascade of the PI3K (phosphoinositide 3-kinase), MAPK (mitogen-activated protein kinase), GSK-3 (glycogen synthase kinase-3), and IDE (insulin-degrading enzyme) pathways. This increases tau hyperphosphorylation. On the other hand, the low expression of glucose transporter (GLUT)-1 and GLUT-3 in the different brain regions (2) is related to the downregulation of hexosamines, which decreases O-GlcNAcylation and increases tau hyperphosphorylation. Moreover, mitochondrial dysfunction (3) caused by functional and structural changes in mitochondria and the production of ROS (4) increase protein aggregation and compromise both the intracellular and membrane components of the neurons. Moreover, mitophagy and autophagy dysfunction (5) also contribute to the development and progression of Alzheimer's disease in type 2 diabetes mellitus. PI3K: Phosphoinositide 3-kinase; MAPK: Mitogen-activated protein kinase; IDE: Insulin-degrading enzyme; GSK-3: Glycogen synthase kinase-3; PSEN1: Presenilin 1; ROS: Reactive oxygen species.

and a lower concentration of insulin in the cerebrospinal fluid (CSF), which shows decreased brain insulin uptake[43]. These findings together with those reported by other studies[44,45], suggest that insulin signaling disruption can be particularly important in the physiopathology of AD in those individuals who are not homozygous for the APOE-ε4 allele.

More recent studies point out that due to its effects on neurodegeneration, brain glucose metabolism, and cognitive performance, peripheral IR could be associated with the pathophysiology of AD in pre-diabetic[46] and non-diabetic patients[47,48]. A study that included 130 non-diabetic patients with AD reported that IR was independently associated with decreased glucose metabolism in the hippocampus and with a lower volume of gray matter[47]. Likewise, a second study reported that high serum insulin levels were significantly associated with severe AD presentations. This significance persisted when non-diabetic patients were excluded[48]. In fact, different studies have shown a decrease in AD incidence[49] and improvement of cognitive performance and brain insulin metabolism in patients with AD that have been treated with insulin or insulin-sensitizing drugs. This provides further evidence of the role of IR in the pathogenesis of AD[15,50,51].

Abnormalities in insulin signaling: Phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways

The terms "type 3 diabetes mellitus" or "brain insulin resistance" have been coined to describe the dysfunction of insulin signaling seen in AD[52]. IR decreases the

availability of glucose needed for neuronal synaptic transmissions due to the deficiency of certain metabolites produced in the glycolytic pathway, such as coenzyme A and succinyl coenzyme A. These are key precursors for the synthesis of acetylcholine, the main neurotransmitter related to cognition[53]. Similarly, it has been proposed that IR induces a series of changes in molecular mechanisms that promote the synthesis and degradation of the amyloid beta peptide and the hyperphosphorylation of the tau protein[54].

Dysfunction in insulin signaling mainly affects the efficiency of the phosphatidylinositol 3-kinase (PI3K). Studies have reported that the brains of patients suffering from AD and T2DM have decreased levels of PI3K, which leads to nervous tissue degeneration. Furthermore, deficient insulin signaling leads to hypoglycemia, which is characteristically found in AD. A decrease in the O-GlcN-acylation of tau has also been observed in the brains of these patients, which is a consequence of hypoglycemia as O-GlcN-acylation is a glucose-dependent process[55,56]. However, this is not the only pathway involved in the pathogenesis of AD. Other factors, such as the mitogen-activated protein kinase (MAPK) pathway, GSK-3, insulin-degrading enzyme (IDE), and microvascular dysfunction, also play an important role in tau hyperphosphorylation. In this sense, a decrease in GSK-3 phosphorylation and increase of its activity can facilitate γ-secretase activities and the processing of the amyloid precursor protein, resulting in higher levels of intracellular amyloid beta peptide[57]. Alternatively, IDE is a zinc metalloprotease that participates in the degradation of different extracellular substrates, such as insulin and amyloid beta. Therefore, its low quantities contribute to an increase in brain amyloid beta levels, especially in the hippocampus [58].

Abnormalities in GLUT-1 and GLUT 3

In regard to GLUTs, clinical studies have revealed that part of the brain hypometabolism in individuals with AD and T2DM may be attributed to a decrease in the expression of GLUT-1 and GLUT-3 in the different areas of the brain[59,60]. Possible causes appear to be post-translational, as no changes have been found in the GLUT-1 ARNm levels in the cerebral cortex[61]. Alterations in glucose transport lead to a reduction in its metabolism, which is associated with the down-regulation of the hexosamine biosynthesis pathway. This involves a decrease in the O-glycosylation of the Ser/Thr residues of tau protein by the β-N-acetylglucosamine (or O-GlcNA cylation), which leads to its abnormal hyperphosphorylation and the formation of NT, contributing to the progression to AD[62].

Moreover, reduced neuronal levels of GLUT-3 have also been identified in patients with T2DM[63]. Likewise, this decrease in the O-GlcNAcylation and hyperphosphorylation of tau has been correlated with decreased levels of GLUT-1 and GLUT-3 in the brain tissue samples of patients with AD[64]. Similarly, other research groups have found a decreased expression of GLUT-1 and/or GLUT-3 in the brain cortex[65] and the dentate gyrus of the hippocampus[66], which were related to the formation of NT. However, the decrease in the expression of these GLUTs, rather than being the main cause of the hypometabolism observed in patients with AD, is more likely to be the result of a decrease in energy demand[64]. Further studies are needed to confirm the direct link between the alterations of GLUTs and neurodegeneration.

Oxidative stress and mitochondrial dysfunction in AD and T2DM

The brain is particularly vulnerable to oxidative damage and mitochondrial dysfunction because of the neurons' high metabolic rate, their dependence on mitochondria to obtain energy, and their low antioxidant defenses[67,68]. Numerous findings have indicated that mitochondrial dysfunction and oxidative stress are implicated in the physiopathology of AD and T2DM[69,70].

Regarding mitochondrial disorders, a decrease in the activity of the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes has been found in the brains of patients with AD. Both complexes are involved in the Krebs cycle, which can lead to mitochondrial dysfunction, glucose hypometabolism, and neuronal oxidative stress[71,72]. Similarly, brain hypometabolism in individuals with AD is related to a disruption in mitochondrial oxidative phosphorylation[73]. Cytochrome c oxidase is the complex with greater alterations in the respiratory chain in neurons from different cortical regions[74-76]. Moreover, mitochondrial dysfunction in AD is associated with an imbalance in mitochondrial fusion and fission, alterations in mitochondrial permeability, calcium homeostasis, and the release of proapoptotic factors[77,78]. Furthermore, the accumulation of amyloid beta deposits in the mitochondria of individuals with AD alters the functions of the respiratory chain and other mitochondrial components, which impacts multiple neuronal properties and activities[79-82].

On the other hand, the increase in ROS and their effects on biomolecules have been associated with the development of cell disorders related to age in AD and DM. Therefore, oxidative stress is one of the main mediators in the processes underlying both diseases[83,84]. Oxidative damage to structural components of the neuronal membrane affects enzymes, ionic channels, and receptors anchored to it. Likewise, it also affects intracellular structures, such as organelles (mitochondria) and DNA or RNA, be it through lipidic peroxidation, nitration, nitrosylation, or carbonylation[85]. Likewise, oxidative stress can lead to the formation of peroxides, carbonyls, advanced glycation end products (AGEs), and advanced oxidation protein products. Furthermore, this results in the denaturalization and aggregation of proteins[86].

The increase in ROS inhibits the cellular production of energy and decreases insulin secretion and sensitivity[87]. Similarly, oxidative damage affects a variety of signaling pathways related to the unfolded protein response and protein degradation, which could lead to IR[88]. Finally, studies have found that mitochondrial disorders related to age increase the oxidative stress in individuals with T2DM, contributing to the development and progression of AD[89-91].

Autophagy in AD and T2DM

Autophagy is a catabolic process which is both constitutive and inducible, wherein a cell uses the liposomal machinery to degrade components or cellular organelles that are damaged or senescent. This process can also recycle biomolecules that are then available for the ensemble of new cell structures[92]. Different studies have reported that autophagy is a crucial deleterious process in AD and T2DM[93-95]. Furthermore, evidence suggests autophagic dysfunction in β-pancreatic cells and other peripheral tissues could contribute to IR[96,97]. This can occur through the deposit of amyloid in the islets, mitochondrial dysfunction, or disorders in regulatory mechanisms, such as the AMPK pathway and the mTOR pathway[69,97-99]. All of these factors are involved in the regulation of autophagy, glucose homeostasis, and peripheral insulin sensitivity.

Moreover, autophagy also intervenes in synaptic plasticity, axonal myelinization, and inflammatory modulation by glial cells. Therefore, alterations in this process contribute to the occurrence and development of neurodegenerative disorders, such as AD[100-102]. This association has been supported by animal and human studies related to AD, reporting alterations in genes and proteins associated with autophagy [95]. Transgenic mice studies have focused on the role of defective lysosomal degradation in the pathogenesis of this condition[103,104]. Studies in patients with AD have reported that mutations in PSEN1 contribute to the defective proteolysis of autophagy substrates[105]. Furthermore, it has been suggested that the excess of autophagy vacuoles reported in dystrophic neurons in such patients is the result of a defective clearance of the autophagosome[106]. Likewise, a significant reduction in the expression of Beclin-1 has been observed in AD patients, which is a fundamental protein in the stages of initiation and maintenance of autophagy, interfering with autophagy activities[107]. Furthermore, dysfunctions in mitophagy have also been identified. This mechanism participates in the detection and elimination of damaged mitochondria. It has also been associated with AD as it induces the deterioration of neuroplasticity by increasing the levels of ROS and decreasing the levels of cellular energy[108,109]. Alterations at the beginning of autophagy, deficient clearance of autophagy substrates, and dysfunction in mitophagy constitute the physiopathological processes important in AD[110].

In synthesis, various associations between T2DM and the development of neurocognitive disorders, specifically AD, have been reported over the years. However, some authors suggest that this association may be confounded by factors, such as smoking, hypertension, APOE E ε4, or brain infarctions, which could explain the progressive emergence of cognitive deterioration and clinical diagnosis of AD in patients with T2DM[111]. At any rate, various epidemiological and postmortem studies (Table 1) have observed the high frequency of AD in patients with T2DM, which could be explained by the different common pathophysiological mechanisms explained in the previous section[112-120].

NEW STRATEGIES FOR THE TREATMENT OF AD

Concerning the multiple pathophysiological links between T2DM and AD, the neuroprotective effects of lifestyle interventions, antidiabetic drugs, and other molecules have gained interest in the scientific community in the past years (Table 2).

Table 1 Epidemiological studies on the link between Alzheimer's disease and type 2 diabetes mellitus

Ref.	Methodology	Results
Gudala *et al*[112]	Meta-analysis with 28 prospective observational studies which evaluated the association between diabetes and the risk of developing AD	A 56% risk of developing AD [RR = 1.56 (95%CI: 1.41-1.73), $P < 0.05$] was reported in patients with diabetes
Profenno *et al*[113]	Meta-analysis of 16 cross-sectional studies evaluating the relationship between diabetes and AD	The presence of diabetes significantly and independently increased the risk of AD [OR = 1.54 (95%CI: 1.33-1.79; $P < 0.001$]
Ohara *et al* [114]	Prospective study that evaluated the association between glucose tolerance status and the development of neurocognitive disorders in 1017 individuals ≥ 60 yr	AD incidence was significantly higher in subjects with T2DM compared to subjects with normal tolerance to glucose [HR = 2.05 (95%CI: 1.18 to 3.57), $P = 0.01$]
Xu *et al* [115]	Prospective study that examined the association between diabetes and the different types of neurocognitive disorders in 1248 older adults. Diagnoses were based on the DSM-III-R criteria	Individuals with non-diagnosed diabetes had a HR of 3.29 (95%CI: 1.20-9.01) $P < 0.05$ for AD diagnosis
Xu *et al* [116]	Prospective study that evaluated the association between T2DM and neurocognitive disorders and AD in 1301 older adults	T2DM diagnosis was significantly associated with neurocognitive disorders [HR = 1.5 (95%CI: 1.0-2.1) $P = 0.04$] and AD [HR = 1.3 (95%CI: 0.9-2.1) $P < 0.05$]
Peila *et al* [117]	Prospective study that examines the association between T2DM and neurocognitive disorder incidence in 2574 Japanese-American men. Diagnosis of neurocognitive disorder was performed through physical exam and MRI according to the NINCDS-ADRDA and DSM-IV criteria	T2DM was significantly associated with AD diagnosis [RR = 1.8 (95%CI: 1.1-2.9) $P < 0.05$]
McIntosh *et al*[118]	Prospective study that examined the relationship between T2DM, biomarkers, and the risk for suffering from neurocognitive disorders in 1289 dementia-free participants. AD biomarker levels were measured from the CSF. Neurocognitive disorders were evaluated through the CDRSB	Untreated diabetic individuals had higher levels of p-tau, p-tau/Aβ1-42, and t-tau/Aβ1-42 in their CSF than normoglycemic or prediabetic individuals ($P < 0.05$). The untreated group did not progress to neurocognitive disorder in higher rates than normoglycemic individuals [HR = 1.602 (95%CI: 1.057-2.429); $P = 0.026$]

AD: Alzheimer's disease; T2DM: Type 2 diabetes mellitus; RR: Relative risk; HR: Hazard ratio; DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders, revised third edition; CSF: Cerebrospinal fluid; CDRSB: Clinical Dementia Rating Sum of Boxes; p-tau: Phosphorylated tau; t-tau: Total tau; Aβ1-42: β-amyloid 1-42; MRI: Magnetic resonance imaging; NINCDS: National Institute of Neurological and Communicative Disorders and Stroke; ADRDA: Alzheimer's Disease and Related Disorders Association; CI: Confidence interval; OR: Odds ratio.

Lifestyle interventions such as nutritional counseling and physical activity are among the first indications made to diabetic and insulin-resistant patients as established by international guidelines[121]. A systematic review by Dunkley *et al*[122] reported the efficacy of these interventions in diabetes prevention, similar to what was observed by a second systematic review in which high-risk T2DM patients participated in lifestyle interventions, observing that there was a lower incidence of T2DM among these subjects[123].

Numerous benefits have also been observed in the context of lifestyle interventions and cognitive decline. As it has been reported, nutritional behaviors such as calorie restriction and the inclusion of an antioxidant-rich diet have shown a beneficial effect in slowing the progression of neurodegenerative illnesses[124]. In addition, dietary interventions with meals characterized by low saturated fat and low glycemic index have shown improvement not only in insulin sensitivity but also in molecular markers of AD, such as an increase of Aβ42 in the CSF, which normally shows reduced levels in patients with AD[125].

Furthermore, when examining the impact of healthy lifestyle behaviors in AD incidence, two longitudinal studies found that there is an additive effect. In consequence, those individuals who practiced two to three behaviors such as following the Mediterranean diet, performing physical activity, and having a low consumption of alcohol showed a lower incidence of AD than those performing only one or none of these behaviors[126]. Overall, lifestyle interventions for diabetic patients, patients with IR, and patients with AD show benefits at the metabolic and cognitive levels and are recommended as part of non-pharmacological treatment and preventive interventions for these conditions.

There are numerous pharmacological interventions for the treatment of T2DM and AD. The systematic administration of insulin has been widely associated with a decrease in the pathological accumulation of amyloid beta as well as with cognitive improvement[127], especially in declarative memory[128,129] and attention[127]. Nevertheless, the use of insulin has not yet proven to be a safe and effective treatment for AD. Furthermore, there are adverse effects such as hypoglycemia[130], which is one of the main issues associated with insulin therapy, related in some cases to higher cardiovascular risk and, subsequently, death[131,132]. Likewise, repeated episodes of

Table 2 Summary of key evidence and ongoing trials on Alzheimer's disease and type 2 diabetes mellitus

Ref.	Antidiabetic drug	Methodology	Results
Craft *et al*[137]	Intranasal insulin	Randomized, double-blind, placebo-controlled trial which evaluated the effects of intranasal insulin administration in 104 adults with amnestic mild cognitive impairment or mild to moderate AD	Treatment with 20 UI of insulin improved delayed memory ($P \leq 0.05$). According to caretakers, a functional improvement was observed in the groups receiving 20 and 40 UI of insulin, respectively ($P \leq 0.01$)
Alp *et al*[164]	Beta-glucan and gliclazide	Preclinical assay including mice with induced diabetes. These were subdivided into six groups among which two groups received treatment with beta-glucan or gliclazide. Different parameters were used to determine the level of oxidative stress, including paraoxonase-1, total antioxidative status, and malondialdehyde	Mice with induced diabetes with no treatment presented high levels of malondialdehyde with a decrease in paraoxonase-1. Groups treated with beta-glucan and gliclazide presented a return of these values to normal levels after treatment, showing a decrease in brain oxidative stress ($P \leq 0.05$)
Mostafa *et al* [174]	Metformin	Preclinical study in mice in which a group received scopolamine and metformin at and the other group received scopolamine and rivastigmine. Malondialdehyde, Akt, phosphorylated Akt, phosphorylated tau, and acetylcholinesterase levels were determined	The functionality of mice receiving scopolamine and a dose of metformin of 100 mg/kg per day was better than the group that was not administered with metformin. They also presented less inflammation and oxidative stress compared with the group receiving rivastigmine. An increase in phosphorylated Akt was observed
Qi *et al*[188]	Liraglutide	Forty mice were divided into four groups. The group with amyloid beta-induced AD was administered with liraglutide for 8 weeks and their cognitive performance was evaluated using a Morris water labyrinth	A protective effect in cognitive performance was observed in mice administered with liraglutide. Likewise, less structural changes in pyramidal neurons were observed, as well as a decrease in tau phosphorylation
Adler *et al* [209]	Amylin	The amylin levels in AD patients, patients with mild cognitive dysfunctions, and the control group were determined. Likewise, pramlintide, an amylin analog, was administered in AD mice in which oxidative stress and cognition were evaluated	Lower levels of amylin in patients with AD and mild cognitive dysfunction were observed compared with the control group. Mice administered with pramlintide showed improvement in cognition and synaptic markers as well as a decrease in oxidative stress in the hippocampus
NCT01843075 [190]	Liraglutide	Multicenter, randomized, double-blind, placebo-controlled Phase IIb study in patients with mild AD	-
NCT03980730 [208]	Azeliragon	Multicenter, randomized, double-blind, placebo-controlled, Phase II/III studies to evaluate the safety and efficacy of azeliragon as a treatment for subjects with mild AD	-
NCT02462161 [142]	Intranasal insulin aspart	Pilot phase I clinical trial that will examine the effects of intranasal insulin aspart on cognition, daily function, blood, and cerebral spinal fluid markers of AD	-
NCT02503501 [143]	Intranasal insulin glulisine	A phase II, single center, randomized, double-blind, placebo-controlled study that will evaluate the safety and effectiveness of intranasal glulisine in patients with probable AD	-

AD: Alzheimer's disease.

hypoglycemia caused by insulin therapy could be involved in the worsening of the cognitive deficit observed in certain patients with DM[133,134]. Therefore, this limits the use of insulin as treatment in patients with AD and other cognitive disorders[135]. Furthermore, pharmacological preparations for insulin injections do not entirely cross the BBB, limiting its distribution in the CNS[136]. Therefore, intranasal insulin administration emerges as a highly viable alternative[137-143].

Although only a few studies have evaluated its short-term effect on healthy individuals[138,139], the chronic administration of intranasal insulin in cognitively normal patients has been associated with higher memory performance. In this context, a study performed by Reger *et al*[140] showed that patients with cognitive defects who were administered with intranasal insulin (20 IU, 2×/d) displayed an improvement in both memory recall and their functional state based on the observations of their caretakers. Similar findings were reported by Benedict *et al*[139] who administered intranasal insulin (3×/d, 40 IU/doses) for 8 wk, which led to an improvement in late words recall. Likewise, a phase II/III clinical assay conducted by Craft *et al*[141] included patients with AD or mild cognitive deficits who were treated with intranasal insulin through a special device (Kurve Technology). Significant score improvements were found at months 15 and 18 (-5.70 and -5.78 points, nominal $P = 0.004$ and 0.018).

Alternatively, leptin and ghrelin are hormones with several functions in energetic balance and have a possible role in the pathophysiology and treatment of AD[144]. Indeed, late-life weight loss and midlife obesity — both of which involve dysfunctions

in leptin signaling — have been associated with a higher risk of developing AD[145]. Midlife obesity is linked to high levels of circulating leptin, which leads to the central resistance to these pathologically high levels. This has been described among obese individuals as a component in IR and T2DM. Meanwhile, late-life weight loss has been related to low circulating levels of leptin[145,146]. Low brain leptin signaling has been found to worsen hippocampus functionality and decrease the neuroprotection against various central processes in the pathogenesis of AD, such as the metabolism of amyloid beta and tau[145,147]. The administration of leptin may renew insulin sensitivity by interacting with the insulin receptor and its signaling pathway[148-150] and by decreasing the pro-inflammatory response[146]. Likewise, in mice with AD, leptin has shown a promising therapeutic effect, improving the formation of memory, synaptic plasticity, and performance in learning activities[151-153].

On the other hand, many researchers have recently focused on evaluating the influence of the ghrelin-insulin system in glucose homeostasis. Ghrelin is unable to improve insulin sensitivity as it is activated with low levels of blood glucose and acts as a counterregulatory hormone[154]. However, it activates a vast number of signaling pathways for growth factors that compensate the loss of insulin signaling[155]. Although a large long-term impact in glycogenic metabolism has not been observed, the administration of ghrelin in AD patients has emerged as a possible therapeutic target by improving the pathological markers of the disease. This could be due to an interaction with the growth hormone secretagogue receptor 1α (GHSR1α). Ghrelin/GHSR1α signaling plays an important role in the synaptic physiology of the hippocampus and memory maintenance through the regulation of the D1 dopamine receptor (DRD1)[155,156]. Current emergent evidence suggests that the disruption of GHSR1α function induces hippocampal stress and memory deficits[157]. Furthermore, animal model studies have reported that the administration of ghrelin or analogs, such as MK0677 and LY444711, can inhibit the accumulation of amyloid beta and the hyperphosphorylation of tau protein by phosphorylating GSK-3β *via* the AMPK and PI3K/Akt pathways[158-160]. This may also reduce oxidative stress, excitotoxicity, and neuroinflammation and improve cognition and memory[158,161,162].

Clinical evidence has shown that the use of certain oral hypoglycemic drugs is associated with a lower risk of dementia[163]. Sulfonylureas have been observed to modulate diabetes-induced oxidative stress[70]. In this context, Alp *et al*[164] reported that the administration of gliclazide can potentiate antioxidant mechanisms and decrease the oxidative index in the brain of diabetic rats. These findings are consistent with that of Baraka and ElGhotny[165] and Abdallah *et al*[166], in which the administration of glibenclamide in mice reduced the hyperphosphorylation of tau and modulation of oxidative stress. However, further research is required to demonstrate the efficacy of sulfonylureas as a possible treatment for AD. In addition, it has been demonstrated that just as insulin therapy, sulfonylureas also have severe hypoglycemic effects[167,168], and repeated hypoglycemia episodes could lead to cognitive alterations or a worsening of cognitive deficits.

Evidence regarding the use of metformin in the treatment of AD has been particularly controversial recently. In theory, it could decrease tau phosphorylation[169] and the interleukin-1β-mediated activation of phosphokinases Akt and MAPK[170]. Moreover, it can inhibit complex 1 of the mitochondrial respiratory chain, inducing an increase in cyclic adenosine monophosphate (cAMP) and activating PKA and AMPK [171,172]. Likewise, a study evaluated the ability of metformin treatment for a year in mice models of AD, reporting a gender-dependent effect, wherein AMPK activation in female mice improved memory and learning, while in male mice memory and cognitive behavior worsened, possibly due to hormonal issues[173]. Furthermore, Mostafa *et al*[174] reported that only low doses of metformin (100 mg/kg) in rats were associated with a delay in memory loss, possibly due to the suppression of Akt. Despite this, a study assessing the risk of AD in patients treated with antidiabetics found that those with long-term metformin treatment were associated with worse cognitive performance[175]. Long-term studies, with a greater number of patients and a standardized methodology, are needed to understand the true role of metformin in AD treatment, as current evidence appears contradictory.

The use of TZD has also been a subject of study for the treatment of AD, as they cause an overexpression of PPARγ in the temporal cortex, which has been associated with a decrease in amyloid beta plaque formation, a decrease in β-secretase levels, and the expression of PPA. Likewise, it modulates calcium homeostasis in the hippocampus, in association with an improvement in cognition[176-178]. Cheng *et al* [179] reported that pioglitazone can improve memory and cognition in the early stages of AD. Similarly, in a pilot study performed in individuals with AD and T2DM, Sato *et al*[180] have found that patients treated with pioglitazone for 6 mo showed cognitive

improvement compared with the placebo group. However, another study in which AD patients without diabetes were treated with TZD for 18 mo did not find any significant cognitive improvement[181]. Currently, an active clinical assay (NCT1931566) with a more extensive sample intends to determine the efficacy of pioglitazone treatment for a longer period in patients with mild cognitive impairment [182].

Since the activation of glucagon-like peptide 1 (GLP-1) receptors and glucose-dependent insulinotropic polypeptides (GIP) in the CNS has been associated with neuroprotective effects[183], the use of GLP-1 and GIP analogs in AD has emerged as a promising therapeutic option. In preclinical studies, the administration of liraglutide in mice has demonstrated to decrease tau hyperphosphorylation and the deposition of amyloid plaque. Likewise, it prevents neuronal loss and the deterioration of synaptic plasticity and promotes beneficial effects on neurogenesis and brain microcir-culation [184-188]. A pilot study that evaluated the administration of liraglutide for 6 mo in AD patients did not find any significant cognitive improvement. However, it was shown that patients treated with GLP-1 agonists had less deterioration of glucose metabolism compared with those treated with placebo[189]. Moreover, a clinical trial assessing the efficacy of liraglutide in a larger group of patients with AD is ongoing[190].

With regard to GIP, different analogs have been able to show improvement in neurodegenerative diseases. Gault and Hölscher[191] reported that the use of D-ala2-GIP and N-glyc-GIP analogs reversed the synaptic plasticity alterations that had been induced by amyloid. D-Ala2-GIP has also been reported to decrease the amyloid plaque load, chronic inflammation, and oxidative stress, improving memory formation and synaptic plasticity as well as normalizing neurogenesis in AD animal models[192, 193]. Based on these findings, the therapeutic effect of novel dual GLP-1/GIP agonists has been evaluated, as well as more recent triple GLP-1/GIP/glucagon agonists[194]. Clear neuroprotective effects have also been observed, reducing inflammation, oxidative stress, and apoptotic signaling and protecting memory formation and synaptic activity[194-196]. On the other hand, the administration of DPP4 inhibitors, such as saxagliptin and vildagliptin, have also been associated with a decrease in amyloid beta deposition, tau phosphorylation, and improvement in memory retention [197,198]. However, these findings are limited to preclinical studies in mice.

Similarly, sodium-glucose transport protein 2 inhibitors (SGLT2i) have been proven to have neuroprotective effects in animal models[199]. This has been reported in obese and diabetic mice, in which positive results on metabolic and brain function parameters have been observed. In addition, the attenuation of physiopathological processes like mitochondrial dysfunction, IR, inflammation, oxidative stress, and apoptosis as well as improvement in cognition, neurogenesis, synaptic density, and synaptic plasticity of the hippocampus has been reported[200-202].

These findings have been recently observed in AD-T2M mice in a study performed by Hierro-Bujalance *et al*[203], in which it was reported that empagliflozin can reduce the density of the senile plaque and the levels of amyloid beta in the brain cortex and hippocampus.

There is a scarce number of studies providing clinical evidence on the use of SGLT2i in AD. In this sense, Wium-Andersen *et al*[204] performed a nested case-control study in which they established that the use of SGLT2i reduces the risk of dementia in diabetic patients (odds ratio of 0.58; 95% confidence interval: 0.42-0.81; $P < 0.05$). Currently, a double-blind, randomized, placebo-controlled, parallel group, 12-wk study is underway. Its goal is to investigate the effect of dapagliflozin in patients who possibly have AD[205].

Azeliragon, a novel drug, has been reported to decrease amyloid deposition and brain inflammation by antagonizing the AGE receptor[206,207]. Based on the preliminary results from a phase III 18-month clinical trial in AD patients, the use of azeliragon decreased pro-inflammatory markers, hippocampus atrophy, and cognitive deterioration. More clinical trials are needed to confirm these findings[208]. Finally, amylin, which can cross the BBB and has effects at the CNS level, has been suggested to have a role in mood disorders as well as neurodegenerative disorders[136]. In AD patients, amylin plasma levels are considerably low, and the administration of analogs, such as pramlintide, has been associated with a decrease in neuroinflammation, oxidative stress, and memory improvement in AD mice[209]. Therefore, its potential use in clinical studies in the upcoming years could be promising.

CONCLUSION

Different studies have demonstrated the existence of a marked association between T2DM and the development of AD. Significant advances in the field of neuroendocrinology have investigated the underlying molecular mechanisms involved in the link between both disorders. Although various confounding factors may intervene in this relationship, studies have found that these diseases may share pathophysiological phenomena, including several abnormalities in insulin signaling in the PI3K and MAPK pathways in the brain tissues as well as the disruption of mitochondrial function, autophagy, GLUTs 1 and 3, and oxidative stress. This overlap leads to new common therapeutic perspectives, and various antidiabetic treatments have been implemented in multiple large-scale clinical and epidemiological studies. However, future clinical trials on the efficacy of these novel therapeutic interventions are needed to better characterize the true scope of this prospect.

REFERENCES

1 **World Health Organization**. Dementia. [cited 21 October 2020]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/dementia
2 **American Diabetes Association**. 12. Older Adults: Standards of Medical Care in Diabetes-2020. *Diabetes Care* 2020; **43**: S152-S162 [PMID: 31862755 DOI: 10.2337/dc20-S012]
3 **International Diabetes Federation**. Worldwide toll of diabetes. [cited 21 October 2020]. In: International Diabetes Federation [Internet]. Available from: https://diabetesatlas.org/en/sections/worldwide-toll-of-diabetes.html
4 **Bermudez V**, Salazar J. Prevalence and Risk Factors associated with Impaired Fasting Glucose in Adults from Maracaibo City, Venezuela. *J Diabetes Metab* 2016; **7** [DOI: 10.4172/2155-6156.1000683]
5 **De La Cruz Vargas JA**, Dos Santos F, Dyzinger W, Herzog S. Medicina del Estilo de Vida: trabajando juntos para revertir la epidemia de las enfermedades crónicas en Latinoamérica. *Cienc E Innov En Salud* 2017; **4**: 1-7 [DOI: 10.17081/innosa.4.2.2870]
6 **Alicic RZ**, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. *Clin J Am Soc Nephrol* 2017; **12**: 2032-2045 [PMID: 28522654 DOI: 10.2215/CJN.11491116]
7 **Yau JW**, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY; Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 2012; **35**: 556-564 [PMID: 22301125 DOI: 10.2337/dc11-1909]
8 **Mirhoseini M**, Saleh N, Momeni A, Deris F, Asadi-Samani M. A study on the association of diabetic dermopathy with nephropathy and retinopathy in patients with type 2 diabetes mellitus. *J Nephropathol* 2016; **5**: 139-143 [PMID: 27921026 DOI: 10.15171/jnp.2016.26]
9 **Thiruvoipati T**, Kielhorn CE, Armstrong EJ. Peripheral artery disease in patients with diabetes: Epidemiology, mechanisms, and outcomes. *World J Diabetes* 2015; **6**: 961-969 [PMID: 26185603 DOI: 10.4239/wjd.v6.i7.961]
10 **Xue M**, Xu W, Ou YN, Cao XP, Tan MS, Tan L, Yu JT. Diabetes mellitus and risks of cognitive impairment and dementia: A systematic review and meta-analysis of 144 prospective studies. *Ageing Res Rev* 2019; **55**: 100944 [PMID: 31430566 DOI: 10.1016/j.arr.2019.100944]
11 **Dá Mesquita S**, Ferreira AC, Sousa JC, Correia-Neves M, Sousa N, Marques F. Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. *Neurosci Biobehav Rev* 2016; **68**: 547-562 [PMID: 27328788 DOI: 10.1016/j.neubiorev.2016.06.014]
12 **Sandhir R**, Gupta S. Molecular and biochemical trajectories from diabetes to Alzheimer's disease: A critical appraisal. *World J Diabetes* 2015; **6**: 1223-1242 [PMID: 26464760 DOI: 10.4239/wjd.v6.i12.1223]
13 **Lee SH**, Zabolotny JM, Huang H, Lee H, Kim YB. Insulin in the nervous system and the mind: Functions in metabolism, memory, and mood. *Mol Metab* 2016; **5**: 589-601 [PMID: 27656397 DOI: 10.1016/j.molmet.2016.06.011]
14 **Samuel VT**, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest* 2016; **126**: 12-22 [PMID: 26727229 DOI: 10.1172/JCI77812]
15 **Craft S**, Claxton A, Baker LD, Hanson AJ, Cholerton B, Trittschuh EH, Dahl D, Caulder E, Neth B, Montine TJ, Jung Y, Maldjian J, Whitlow C, Friedman S. Effects of Regular and Long-Acting Insulin on Cognition and Alzheimer's Disease Biomarkers: A Pilot Clinical Trial. *J Alzheimers Dis* 2017; **57**: 1325-1334 [PMID: 28372335 DOI: 10.3233/JAD-161256]
16 **Koepsell H**. Glucose transporters in brain in health and disease. *Pflugers Arch* 2020; **472**: 1299-

1343 [PMID: 32789766 DOI: 10.1007/s00424-020-02441-x]
17 **Mergenthaler P**, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends Neurosci* 2013; **36**: 587-597 [PMID: 23968694 DOI: 10.1016/j.tins.2013.07.001]
18 **McAllister MS**, Krizanac-Bengez L, Macchia F, Naftalin RJ, Pedley KC, Mayberg MR, Marroni M, Leaman S, Stanness KA, Janigro D. Mechanisms of glucose transport at the blood-brain barrier: an *in vitro* study. *Brain Res* 2001; **904**: 20-30 [PMID: 11516408 DOI: 10.1016/s0006-8993(01)02418-0]
19 **Bermudez V**, Bermudez F, Arraiz N, Leal E, Linares S, Mengual E, Valdelamar L, Seyfi H, Amell A, Carrillo M, Silva C. Biología molecular de los transportadores de glucosa: clasificación, estructura y distribución. *Arch Venez Farmacol Ter* 2007; **26**: 76-86
20 **Jurcovicova J**. Glucose transport in brain - effect of inflammation. *Endocr Regul* 2014; **48**: 35-48 [PMID: 24524374 DOI: 10.4149/endo_2014_01_35]
21 **Qutub AA**, Hunt CA. Glucose transport to the brain: a systems model. *Brain Res Brain Res Rev* 2005; **49**: 595-617 [PMID: 16269321 DOI: 10.1016/j.brainresrev.2005.03.002]
22 **Bedse G**, Di Domenico F, Serviddio G, Cassano T. Aberrant insulin signaling in Alzheimer's disease: current knowledge. *Front Neurosci* 2015; **9**: 204 [PMID: 26136647 DOI: 10.3389/fnins.2015.00204]
23 **Blázquez E**, Velázquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front Endocrinol (Lausanne)* 2014; **5**: 161 [PMID: 25346723 DOI: 10.3389/fendo.2014.00161]
24 **Rojas J**, Bermúdez V, Leal E, Cano R, Luti Y, Acosta L, Finol F, AparicioD, Arraiz N, Linares S, Rojas E, Canelón R, Deisiree S. Insulinorresistencia e hiperinsulinemia como factores de riesgo para enfermedad cardiovascular. *Arch Venez Farmacol Ter* 2008; **27**: 30-40
25 **Baranowska-Bik A**, Bik W. Insulin and brain aging. *Prz Menopauzalny* 2017; **16**: 44-46 [PMID: 28721128 DOI: 10.5114/pm.2017.68590]
26 **Tascone LDS**, Bottino CMC. Neurobiology of neuropsychiatric symptoms in Alzheimer's disease: A critical review with a focus on neuroimaging. *Dement Neuropsychol* 2013; **7**: 236-243 [PMID: 29213845 DOI: 10.1590/S1980-57642013DN70300002]
27 **Narvaez E**, Pelaez J, Almeida K, Alvarez C, Mendoza C, Morales A, Godos D, Del Salto Ocaña T, Catota M. Implication of apolipoprotein e polymorphisms in the atherosclerosis and Alzheimer's disease physiopathology. *Rev Latinoam Hipertens* 2018; **13**: 97-102
28 **Vanegas H**. Buscando las bases moleculares de la enfermedad de Alzheimer. *Gac Méd Caracas* 2017; **125**: 4-11
29 **Gilbert BJ**. The role of amyloid β in the pathogenesis of Alzheimer's disease. *J Clin Pathol* 2013; **66**: 362-366 [PMID: 23526599 DOI: 10.1136/jclinpath-2013-201515]
30 **Yu JT**, Tan L, Hardy J. Apolipoprotein E in Alzheimer's disease: an update. *Annu Rev Neurosci* 2014; **37**: 79-100 [PMID: 24821312 DOI: 10.1146/annurev-neuro-071013-014300]
31 **Sadigh-Eteghad S**, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. *Med Princ Pract* 2015; **24**: 1-10 [PMID: 25471398 DOI: 10.1159/000369101]
32 **Varma VR**, Varma S, An Y, Hohman TJ, Seddighi S, Casanova R, Beri A, Dammer EB, Seyfried NT, Pletnikova O, Moghekar A, Wilson MR, Lah JJ, O'Brien RJ, Levey AI, Troncoso JC, Albert MS, Thambisetty M. Alpha-2 macroglobulin in Alzheimer's disease: a marker of neuronal injury through the RCAN1 pathway. *Mol Psychiatry* 2017; **22**: 13-23 [PMID: 27872486 DOI: 10.1038/mp.2016.206]
33 **Kumar A**, Singh A, Ekavali. A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol Rep* 2015; **67**: 195-203 [PMID: 25712639 DOI: 10.1016/j.pharep.2014.09.004]
34 **Hasegawa M**. Molecular Mechanisms in the Pathogenesis of Alzheimer's disease and Tauopathies-Prion-Like Seeded Aggregation and Phosphorylation. *Biomolecules* 2016; **6** [PMID: 27136595 DOI: 10.3390/biom6020024]
35 **Barbier P**, Zejneli O, Martinho M, Lasorsa A, Belle V, Smet-Nocca C, Tsvetkov PO, Devred F, Landrieu I. Role of Tau as a Microtubule-Associated Protein: Structural and Functional Aspects. *Front Aging Neurosci* 2019; **11**: 204 [PMID: 31447664 DOI: 10.3389/fnagi.2019.00204]
36 **Chesser AS**, Pritchard SM, Johnson GV. Tau clearance mechanisms and their possible role in the pathogenesis of Alzheimer disease. *Front Neurol* 2013; **4**: 122 [PMID: 24027553 DOI: 10.3389/fneur.2013.00122]
37 **Šimić G**, Babić Leko M, Wray S, Harrington C, Delalle I, Jovanov-Milošević N, Bažadona D, Buée L, de Silva R, Di Giovanni G, Wischik C, Hof PR. Tau Protein Hyperphosphorylation and Aggregation in Alzheimer's Disease and Other Tauopathies, and Possible Neuroprotective Strategies. *Biomolecules* 2016; **6**: 6 [PMID: 26751493 DOI: 10.3390/biom6010006]
38 **Tsartsalis S**, Xekardaki A, Hof PR, Kövari E, Bouras C. Early Alzheimer-type lesions in cognitively normal subjects. *Neurobiol Aging* 2018; **62**: 34-44 [PMID: 29107845 DOI: 10.1016/j.neurobiolaging.2017.10.002]
39 **Sonne DP**, Hemmingsen B. Comment on American Diabetes Association. *Standards of Medical Care in Diabetes-2017*. Diabetes Care 2017;40(Suppl. 1):S1-S135. *Diabetes Care* 2017; **40**: e92-e93 [PMID: 28637892 DOI: 10.2337/dc17-0299]

40 **Jayaraman A**, Pike CJ. Alzheimer's disease and type 2 diabetes: multiple mechanisms contribute to interactions. *Curr Diab Rep* 2014; **14**: 476 [PMID: 24526623 DOI: 10.1007/s11892-014-0476-2]

41 **Claxton A**, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, Callaghan M, Arbuckle M, Behl C, Craft S. Long-acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. *J Alzheimers Dis* 2015; **44**: 897-906 [PMID: 25374101 DOI: 10.3233/JAD-141791]

42 **Frölich L**, Blum-Degen D, Bernstein HG, Engelsberger S, Humrich J, Laufer S, Muschner D, Thalheimer A, Türk A, Hoyer S, Zöchling R, Boissl KW, Jellinger K, Riederer P. Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J Neural Transm (Vienna)* 1998; **105**: 423-438 [PMID: 9720972 DOI: 10.1007/s007020050068]

43 **Craft S**, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D Jr. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology* 1998; **50**: 164-168 [PMID: 9443474 DOI: 10.1212/wnl.50.1.164]

44 **Craft S**, Asthana S, Schellenberg G, Baker L, Cherrier M, Boyt AA, Martins RN, Raskind M, Peskind E, Plymate S. Insulin effects on glucose metabolism, memory, and plasma amyloid precursor protein in Alzheimer's disease differ according to apolipoprotein-E genotype. *Ann N Y Acad Sci* 2000; **903**: 222-228 [PMID: 10818510 DOI: 10.1111/j.1749-6632.2000.tb06371.x]

45 **Craft S**, Asthana S, Cook DG, Baker LD, Cherrier M, Purganan K, Wait C, Petrova A, Latendresse S, Watson GS, Newcomer JW, Schellenberg GD, Krohn AJ. Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer's disease: interactions with apolipoprotein E genotype. *Psychoneuroendocrinology* 2003; **28**: 809-822 [PMID: 12812866 DOI: 10.1016/s0306-4530(02)00087-2]

46 **Baker LD**, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol* 2011; **68**: 51-57 [PMID: 20837822 DOI: 10.1001/archneurol.2010.225]

47 **Femminella GD**, Livingston NR, Raza S, van der Doef T, Frangou E, Love S, Busza G, Calsolaro V, Carver S, Holmes C, Ritchie CW, Lawrence RM, McFarlane B, Tadros G, Ridha BH, Bannister C, Walker Z, Archer H, Coulthard E, Underwood B, Prasanna A, Koranteng P, Karim S, Junaid K, McGuinness B, Passmore AP, Nilforooshan R, Macharouthu A, Donaldson A, Thacker S, Russell G, Malik N, Mate V, Knight L, Kshemendran S, Tan T, Holscher C, Harrison J, Brooks DJ, Ballard C, Edison P. Does insulin resistance influence neurodegeneration in non-diabetic Alzheimer's subjects? *Alzheimers Res Ther* 2021; **13**: 47 [PMID: 33597002 DOI: 10.1186/s13195-021-00784-w]

48 **Thankappan S**, Sen S, Subramanian S, Sinha P, Purushottam M, Bharath S. Insulin resistance in patients with Alzheimer's dementia: A controlled study from India. *Asian J Psychiatr* 2018; **38**: 33-34 [PMID: 30391679 DOI: 10.1016/j.ajp.2018.10.026]

49 **Ye F**, Luo YJ, Xiao J, Yu NW, Yi G. Impact of Insulin Sensitizers on the Incidence of Dementia: A Meta-Analysis. *Dement Geriatr Cogn Disord* 2016; **41**: 251-260 [PMID: 27250528 DOI: 10.1159/000445941]

50 **Watson KT**, Wroolie TE, Tong G, Foland-Ross LC, Frangou S, Singh M, McIntyre RS, Roat-Shumway S, Myoraku A, Reiss AL, Rasgon NL. Neural correlates of liraglutide effects in persons at risk for Alzheimer's disease. *Behav Brain Res* 2019; **356**: 271-278 [PMID: 30099030 DOI: 10.1016/j.bbr.2018.08.006]

51 **Koenig AM**, Mechanic-Hamilton D, Xie SX, Combs MF, Cappola AR, Xie L, Detre JA, Wolk DA, Arnold SE. Effects of the Insulin Sensitizer Metformin in Alzheimer Disease: Pilot Data From a Randomized Placebo-controlled Crossover Study. *Alzheimer Dis Assoc Disord* 2017; **31**: 107-113 [PMID: 28538088 DOI: 10.1097/WAD.0000000000000202]

52 **Kandimalla R**, Thirumala V, Reddy PH. Is Alzheimer's disease a Type 3 Diabetes? *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**: 1078-1089 [PMID: 27567931 DOI: 10.1016/j.bbadis.2016.08.018]

53 **De Felice FG**, Lourenco MV, Ferreira ST. How does brain insulin resistance develop in Alzheimer's disease? *Alzheimers Dement* 2014; **10**: S26-S32 [PMID: 24529521 DOI: 10.1016/j.jalz.2013.12.004]

54 **Ahmad W**. Overlapped metabolic and therapeutic links between Alzheimer and diabetes. *Mol Neurobiol* 2013; **47**: 399-424 [PMID: 23011810 DOI: 10.1007/s12035-012-8352-z]

55 **De Felice FG**, Ferreira ST. Inflammation, defective insulin signaling, and mitochondrial dysfunction as common molecular denominators connecting type 2 diabetes to Alzheimer disease. *Diabetes* 2014; **63**: 2262-2272 [PMID: 24931033 DOI: 10.2337/db13-1954]

56 **Ansari SA**, Emerald BS. The Role of Insulin Resistance and Protein O-GlcNAcylation in Neurodegeneration. *Front Neurosci* 2019; **13**: 473 [PMID: 31143098 DOI: 10.3389/fnins.2019.00473]

57 **Mravec B**, Horvathova L, Padova A. Brain Under Stress and Alzheimer's Disease. *Cell Mol Neurobiol* 2018; **38**: 73-84 [PMID: 28699112 DOI: 10.1007/s10571-017-0521-1]

58 **Simó R**, Ciudin A, Simó-Servat O, Hernández C. Cognitive impairment and dementia: a new emerging complication of type 2 diabetes-The diabetologist's perspective. *Acta Diabetol* 2017; **54**: 417-424 [PMID: 28210868 DOI: 10.1007/s00592-017-0970-5]

59 **Shah K**, Desilva S, Abbruscato T. The role of glucose transporters in brain disease: diabetes and Alzheimer's Disease. *Int J Mol Sci* 2012; **13**: 12629-12655 [PMID: 23202918 DOI: 10.3390/ijms131012629]

60 **Chen Z**, Zhong C. Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. *Prog Neurobiol* 2013; **108**: 21-43 [PMID: 23850509 DOI: 10.1016/j.pneurobio.2013.06.004]

61 **Mooradian AD**, Chung HC, Shah GN. GLUT-1 expression in the cerebra of patients with Alzheimer's disease. *Neurobiol Aging* 1997; **18**: 469-474 [PMID: 9390772 DOI: 10.1016/s0197-4580(97)00111-5]

62 **Liu F**, Shi J, Tanimukai H, Gu J, Grundke-Iqbal I, Iqbal K, Gong CX. Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. *Brain* 2009; **132**: 1820-1832 [PMID: 19451179 DOI: 10.1093/brain/awp099]

63 **Liu Y**, Liu F, Grundke-Iqbal I, Iqbal K, Gong CX. Brain glucose transporters, O-GlcNAcylation and phosphorylation of tau in diabetes and Alzheimer's disease. *J Neurochem* 2009; **111**: 242-249 [PMID: 19659459 DOI: 10.1111/j.1471-4159.2009.06320.x]

64 **Liu Y**, Liu F, Iqbal K, Grundke-Iqbal I, Gong CX. Decreased glucose transporters correlate to abnormal hyperphosphorylation of tau in Alzheimer disease. *FEBS Lett* 2008; **582**: 359-364 [PMID: 18174027 DOI: 10.1016/j.febslet.2007.12.035]

65 **Simpson IA**, Chundu KR, Davies-Hill T, Honer WG, Davies P. Decreased concentrations of GLUT1 and GLUT3 glucose transporters in the brains of patients with Alzheimer's disease. *Ann Neurol* 1994; **35**: 546-551 [PMID: 8179300 DOI: 10.1002/ana.410350507]

66 **Harr SD**, Simonian NA, Hyman BT. Functional alterations in Alzheimer's disease: decreased glucose transporter 3 immunoreactivity in the perforant pathway terminal zone. *J Neuropathol Exp Neurol* 1995; **54**: 38-41 [PMID: 7815078]

67 **Kann O**, Kovács R. Mitochondria and neuronal activity. *Am J Physiol Cell Physiol* 2007; **292**: C641-C657 [PMID: 17092996 DOI: 10.1152/ajpcell.00222.2006]

68 **Nunomura A**, Honda K, Takeda A, Hirai K, Zhu X, Smith MA, Perry G. Oxidative damage to RNA in neurodegenerative diseases. *J Biomed Biotechnol* 2006; **2006**: 82323 [PMID: 17047315 DOI: 10.1155/JBB/2006/82323]

69 **Correia SC**, Santos RX, Carvalho C, Cardoso S, Candeias E, Santos MS, Oliveira CR, Moreira PI. Insulin signaling, glucose metabolism and mitochondria: major players in Alzheimer's disease and diabetes interrelation. *Brain Res* 2012; **1441**: 64-78 [PMID: 22290178 DOI: 10.1016/j.brainres.2011.12.063]

70 **Butterfield DA**, Di Domenico F, Barone E. Elevated risk of type 2 diabetes for development of Alzheimer disease: a key role for oxidative stress in brain. *Biochim Biophys Acta* 2014; **1842**: 1693-1706 [PMID: 24949886 DOI: 10.1016/j.bbadis.2014.06.010]

71 **Sorbi S**, Bird ED, Blass JP. Decreased pyruvate dehydrogenase complex activity in Huntington and Alzheimer brain. *Ann Neurol* 1983; **13**: 72-78 [PMID: 6219611 DOI: 10.1002/ana.410130116]

72 **Butterworth RF**, Besnard AM. Thiamine-dependent enzyme changes in temporal cortex of patients with Alzheimer's disease. *Metab Brain Dis* 1990; **5**: 179-184 [PMID: 2087217 DOI: 10.1007/BF00997071]

73 **Yao J**, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2009; **106**: 14670-14675 [PMID: 19667196 DOI: 10.1073/pnas.0903563106]

74 **Hirai K**, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci* 2001; **21**: 3017-3023 [PMID: 11312286 DOI: 10.1523/JNEUROSCI.21-09-03017.2001]

75 **Parker WD Jr**, Parks JK. Cytochrome c oxidase in Alzheimer's disease brain: purification and characterization. *Neurology* 1995; **45**: 482-486 [PMID: 7898701 DOI: 10.1212/wnl.45.3.482]

76 **Sims NR**, Finegan JM, Blass JP, Bowen DM, Neary D. Mitochondrial function in brain tissue in primary degenerative dementia. *Brain Res* 1987; **436**: 30-38 [PMID: 3690351 DOI: 10.1016/0006-8993(87)91553-8]

77 **Bonda DJ**, Wang X, Perry G, Smith MA, Zhu X. Mitochondrial dynamics in Alzheimer's disease: opportunities for future treatment strategies. *Drugs Aging* 2010; **27**: 181-192 [PMID: 20210366 DOI: 10.2165/11532140-000000000-00000]

78 **Lloret A**, Badía MC, Mora NJ, Ortega A, Pallardó FV, Alonso MD, Atamna H, Viña J. Gender and age-dependent differences in the mitochondrial apoptogenic pathway in Alzheimer's disease. *Free Radic Biol Med* 2008; **44**: 2019-2025 [PMID: 18387371 DOI: 10.1016/j.freeradbiomed.2008.02.017]

79 **Devi L**, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J Neurosci* 2006; **26**: 9057-9068 [PMID: 16943564 DOI: 10.1523/JNEUROSCI.1469-06.2006]

80 **Cardoso SM**, Santos S, Swerdlow RH, Oliveira CR. Functional mitochondria are required for amyloid beta-mediated neurotoxicity. *FASEB J* 2001; **15**: 1439-1441 [PMID: 11387250 DOI: 10.1096/fj.00-0561fje]

81 **Du H**, Guo L, Fang F, Chen D, Sosunov AA, McKhann GM, Yan Y, Wang C, Zhang H, Molkentin JD, Gunn-Moore FJ, Vonsattel JP, Arancio O, Chen JX, Yan SD. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nat Med* 2008; **14**: 1097-1105 [PMID: 18806802 DOI: 10.1038/nm.1868]

82 **Lustbader JW**, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S,

Chaney M, Trinchese F, Liu S, Gunn-Moore F, Lue LF, Walker DG, Kuppusamy P, Zewier ZL, Arancio O, Stern D, Yan SS, Wu H. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* 2004; **304**: 448-452 [PMID: 15087549 DOI: 10.1126/science.1091230]

83 **Di Domenico F**, Perluigi M, Butterfield DA, Cornelius C, Calabrese V. Oxidative damage in rat brain during aging: interplay between energy and metabolic key target proteins. *Neurochem Res* 2010; **35**: 2184-2192 [PMID: 20963486 DOI: 10.1007/s11064-010-0295-z]

84 **Domínguez RO**, Marschoff ER, González SE, Repetto MG, Serra JA. Type 2 diabetes and/or its treatment leads to less cognitive impairment in Alzheimer's disease patients. *Diabetes Res Clin Pract* 2012; **98**: 68-74 [PMID: 22658669 DOI: 10.1016/j.diabres.2012.05.013]

85 **Butterfield DA**, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. *Free Radic Biol Med* 2002; **32**: 1050-1060 [PMID: 12031889 DOI: 10.1016/s0891-5849(02)00794-3]

86 **Butterfield DA**, Gu L, Di Domenico F, Robinson RA. Mass spectrometry and redox proteomics: applications in disease. *Mass Spectrom Rev* 2014; **33**: 277-301 [PMID: 24930952 DOI: 10.1002/mas.21374]

87 **Moreira PI**, Santos MS, Seiça R, Oliveira CR. Brain mitochondrial dysfunction as a link between Alzheimer's disease and diabetes. *J Neurol Sci* 2007; **257**: 206-214 [PMID: 17316694 DOI: 10.1016/j.jns.2007.01.017]

88 **Maiese K**, Chong ZZ, Shang YC. Mechanistic insights into diabetes mellitus and oxidative stress. *Curr Med Chem* 2007; **14**: 1729-1738 [PMID: 17627510 DOI: 10.2174/092986707781058968]

89 **de la Monte SM**. Insulin resistance and Alzheimer's disease. *BMB Rep* 2009; **42**: 475-481 [PMID: 19712582 DOI: 10.5483/bmbrep.2009.42.8.475]

90 **de la Monte SM**, Tong M. Brain metabolic dysfunction at the core of Alzheimer's disease. *Biochem Pharmacol* 2014; **88**: 548-559 [PMID: 24380887 DOI: 10.1016/j.bcp.2013.12.012]

91 **Orth M**, Schapira AH. Mitochondria and degenerative disorders. *Am J Med Genet* 2001; **106**: 27-36 [PMID: 11579422 DOI: 10.1002/ajmg.1425]

92 **Klionsky DJ**. The molecular machinery of autophagy: unanswered questions. *J Cell Sci* 2005; **118**: 7-18 [PMID: 15615779 DOI: 10.1242/jcs.01620]

93 **Gonzalez CD**, Lee MS, Marchetti P, Pietropaolo M, Towns R, Vaccaro MI, Watada H, Wiley JW. The emerging role of autophagy in the pathophysiology of diabetes mellitus. *Autophagy* 2011; **7**: 2-11 [PMID: 20935516 DOI: 10.4161/auto.7.1.13044]

94 **Caberlotto L**, Nguyen TP, Lauria M, Priami C, Rimondini R, Maioli S, Cedazo-Minguez A, Sita G, Morroni F, Corsi M, Carboni L. Cross-disease analysis of Alzheimer's disease and type-2 Diabetes highlights the role of autophagy in the pathophysiology of two highly comorbid diseases. *Sci Rep* 2019; **9**: 3965 [PMID: 30850634 DOI: 10.1038/s41598-019-39828-5]

95 **Whyte LS**, Lau AA, Hemsley KM, Hopwood JJ, Sargeant TJ. Endo-lysosomal and autophagic dysfunction: a driving factor in Alzheimer's disease? *J Neurochem* 2017; **140**: 703-717 [PMID: 28027395 DOI: 10.1111/jnc.13935]

96 **Barlow AD**, Thomas DC. Autophagy in diabetes: β-cell dysfunction, insulin resistance, and complications. *DNA Cell Biol* 2015; **34**: 252-260 [PMID: 25665094 DOI: 10.1089/dna.2014.2755]

97 **Lee YH**, Kim J, Park K, Lee MS. β-cell autophagy: Mechanism and role in β-cell dysfunction. *Mol Metab* 2019; **27S**: S92-S103 [PMID: 31500836 DOI: 10.1016/j.molmet.2019.06.014]

98 **Herzig S**, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol* 2018; **19**: 121-135 [PMID: 28974774 DOI: 10.1038/nrm.2017.95]

99 **Saxton RA**, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 2017; **168**: 960-976 [PMID: 28283069 DOI: 10.1016/j.cell.2017.02.004]

100 **Lee JA**. Neuronal autophagy: a housekeeper or a fighter in neuronal cell survival? *Exp Neurobiol* 2012; **21**: 1-8 [PMID: 22438673 DOI: 10.5607/en.2012.21.1.1]

101 **Frake RA**, Ricketts T, Menzies FM, Rubinsztein DC. Autophagy and neurodegeneration. *J Clin Invest* 2015; **125**: 65-74 [PMID: 25654552 DOI: 10.1172/JCI73944]

102 **Harris H**, Rubinsztein DC. Control of autophagy as a therapy for neurodegenerative disease. *Nat Rev Neurol* 2011; **8**: 108-117 [PMID: 22187000 DOI: 10.1038/nrneurol.2011.200]

103 **Sun B**, Zhou Y, Halabisky B, Lo I, Cho SH, Mueller-Steiner S, Devidze N, Wang X, Grubb A, Gan L. Cystatin C-cathepsin B axis regulates amyloid beta levels and associated neuronal deficits in an animal model of Alzheimer's disease. *Neuron* 2008; **60**: 247-257 [PMID: 18957217 DOI: 10.1016/j.neuron.2008.10.001]

104 **Yang DS**, Stavrides P, Mohan PS, Kaushik S, Kumar A, Ohno M, Schmidt SD, Wesson D, Bandyopadhyay U, Jiang Y, Pawlik M, Peterhoff CM, Yang AJ, Wilson DA, St George-Hyslop P, Westaway D, Mathews PM, Levy E, Cuervo AM, Nixon RA. Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. *Brain* 2011; **134**: 258-277 [PMID: 21186265 DOI: 10.1093/brain/awq341]

105 **Lee JH**, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, Wolfe DM, Martinez-Vicente M, Massey AC, Sovak G, Uchiyama Y, Westaway D, Cuervo AM, Nixon RA. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell* 2010; **141**: 1146-1158 [PMID: 20541250 DOI: 10.1016/j.cell.2010.05.008]

106 **Boland B**, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, Nixon RA. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. *J Neurosci* 2008; **28**: 6926-6937 [PMID: 18596167 DOI: 10.1523/JNEUROSCI.0800-08.2008]

107 **Pickford F**, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B, Rockenstein E, Levine B, Wyss-Coray T. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *J Clin Invest* 2008; **118**: 2190-2199 [PMID: 18497889 DOI: 10.1172/JCI33585]

108 **Correia SC**, Perry G, Moreira PI. Mitochondrial traffic jams in Alzheimer's disease - pinpointing the roadblocks. *Biochim Biophys Acta* 2016; **1862**: 1909-1917 [PMID: 27460705 DOI: 10.1016/j.bbadis.2016.07.010]

109 **Kerr JS**, Adriaanse BA, Greig NH, Mattson MP, Cader MZ, Bohr VA, Fang EF. Mitophagy and Alzheimer's Disease: Cellular and Molecular Mechanisms. *Trends Neurosci* 2017; **40**: 151-166 [PMID: 28190529 DOI: 10.1016/j.tins.2017.01.002]

110 **Nixon RA**, Yang DS. Autophagy failure in Alzheimer's disease--locating the primary defect. *Neurobiol Dis* 2011; **43**: 38-45 [PMID: 21296668 DOI: 10.1016/j.nbd.2011.01.021]

111 **Vagelatos NT**, Eslick GD. Type 2 diabetes as a risk factor for Alzheimer's disease: the confounders, interactions, and neuropathology associated with this relationship. *Epidemiol Rev* 2013; **35**: 152-160 [PMID: 23314404 DOI: 10.1093/epirev/mxs012]

112 **Gudala K**, Bansal D, Schifano F, Bhansali A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. *J Diabetes Investig* 2013; **4**: 640-650 [PMID: 24843720 DOI: 10.1111/jdi.12087]

113 **Profenno LA**, Porsteinsson AP, Faraone SV. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. *Biol Psychiatry* 2010; **67**: 505-512 [PMID: 19358976 DOI: 10.1016/j.biopsych.2009.02.013]

114 **Ohara T**, Doi Y, Ninomiya T, Hirakawa Y, Hata J, Iwaki T, Kanba S, Kiyohara Y. Glucose tolerance status and risk of dementia in the community: the Hisayama study. *Neurology* 2011; **77**: 1126-1134 [PMID: 21931106 DOI: 10.1212/WNL.0b013e31822f0435]

115 **Xu WL**, von Strauss E, Qiu CX, Winblad B, Fratiglioni L. Uncontrolled diabetes increases the risk of Alzheimer's disease: a population-based cohort study. *Diabetologia* 2009; **52**: 1031-1039 [PMID: 19280172 DOI: 10.1007/s00125-009-1323-x]

116 **Xu WL**, Qiu CX, Wahlin A, Winblad B, Fratiglioni L. Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. *Neurology* 2004; **63**: 1181-1186 [PMID: 15477535 DOI: 10.1212/01.wnl.0000140291.86406.d1]

117 **Peila R**, Rodriguez BL, Launer LJ; Honolulu-Asia Aging Study. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes* 2002; **51**: 1256-1262 [PMID: 11916953 DOI: 10.2337/diabetes.51.4.1256]

118 **McIntosh EC**, Nation DA; Alzheimer's Disease Neuroimaging Initiative. Importance of Treatment Status in Links Between Type 2 Diabetes and Alzheimer's Disease. *Diabetes Care* 2019; **42**: 972-979 [PMID: 30833374 DOI: 10.2337/dc18-1399]

119 **Ahtiluoto S**, Polvikoski T, Peltonen M, Solomon A, Tuomilehto J, Winblad B, Sulkava R, Kivipelto M. Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study. *Neurology* 2010; **75**: 1195-1202 [PMID: 20739645 DOI: 10.1212/WNL.0b013e3181f4d7f8]

120 **Matsuzaki T**, Sasaki K, Tanizaki Y, Hata J, Fujimi K, Matsui Y, Sekita A, Suzuki SO, Kanba S, Kiyohara Y, Iwaki T. Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama study. *Neurology* 2010; **75**: 764-770 [PMID: 20739649 DOI: 10.1212/WNL.0b013e3181eee25f]

121 **American Diabetes Association**. . 5. Lifestyle Management: *Standards of Medical Care in Diabetes-2019*. *Diabetes Care* 2019; **42**: S46-S60 [PMID: 30559231 DOI: 10.2337/dc19-S005]

122 **Dunkley AJ**, Bodicoat DH, Greaves CJ, Russell C, Yates T, Davies MJ, Khunti K. Diabetes prevention in the real world: effectiveness of pragmatic lifestyle interventions for the prevention of type 2 diabetes and of the impact of adherence to guideline recommendations: a systematic review and meta-analysis. *Diabetes Care* 2014; **37**: 922-933 [PMID: 24652723 DOI: 10.2337/dc13-2195]

123 **Schellenberg ES**, Dryden DM, Vandermeer B, Ha C, Korownyk C. Lifestyle interventions for patients with and at risk for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 2013; **159**: 543-551 [PMID: 24126648 DOI: 10.7326/0003-4819-159-8-201310150-00007]

124 **Bhatti GK**, Reddy AP, Reddy PH, Bhatti JS. Lifestyle Modifications and Nutritional Interventions in Aging-Associated Cognitive Decline and Alzheimer's Disease. *Front Aging Neurosci* 2019; **11**: 369 [PMID: 31998117 DOI: 10.3389/fnagi.2019.00369]

125 **Bayer-Carter JL**, Green PS, Montine TJ, VanFossen B, Baker LD, Watson GS, Bonner LM, Callaghan M, Leverenz JB, Walter BK, Tsai E, Plymate SR, Postupna N, Wilkinson CW, Zhang J, Lampe J, Kahn SE, Craft S. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. *Arch Neurol* 2011; **68**: 743-752 [PMID: 21670398 DOI: 10.1001/archneurol.2011.125]

126 **Dhana K**, Evans DA, Rajan KB, Bennett DA, Morris MC. Healthy lifestyle and the risk of Alzheimer dementia: Findings from 2 Longitudinal studies. *Neurology* 2020; **95**: e374-e383 [PMID: 32554763 DOI: 10.1212/WNL.0000000000009816]

127 **Kern W**, Peters A, Fruehwald-Schultes B, Deininger E, Born J, Fehm HL. Improving influence of insulin on cognitive functions in humans. *Neuroendocrinology* 2001; **74**: 270-280 [PMID: 11598383 DOI: 10.1159/000054694]

128 **Craft S**, Asthana S, Newcomer JW, Wilkinson CW, Matos IT, Baker LD, Cherrier M, Lofgreen C, Latendresse S, Petrova A, Plymate S, Raskind M, Grimwood K, Veith RC. Enhancement of memory in Alzheimer disease with insulin and somatostatin, but not glucose. *Arch Gen Psychiatry* 1999; **56**:

1135-1140 [PMID: 10591291 DOI: 10.1001/archpsyc.56.12.1135]
129 **Craft S**, Newcomer J, Kanne S, Dagogo-Jack S, Cryer P, Sheline Y, Luby J, Dagogo-Jack A, Alderson A. Memory improvement following induced hyperinsulinemia in Alzheimer's disease. *Neurobiol Aging* 1996; **17**: 123-130 [PMID: 8786794 DOI: 10.1016/0197-4580(95)02002-0]
130 **McCall AL**. Insulin therapy and hypoglycemia. *Endocrinol Metab Clin North Am* 2012; **41**: 57-87 [PMID: 22575407 DOI: 10.1016/j.ecl.2012.03.001]
131 **Herman ME**, O'Keefe JH, Bell DSH, Schwartz SS. Insulin Therapy Increases Cardiovascular Risk in Type 2 Diabetes. *Prog Cardiovasc Dis* 2017; **60**: 422-434 [PMID: 28958751 DOI: 10.1016/j.pcad.2017.09.001]
132 **Margolis DJ**, Hoffstad O, Strom BL. Association between serious ischemic cardiac outcomes and medications used to treat diabetes. *Pharmacoepidemiol Drug Saf* 2008; **17**: 753-759 [PMID: 18613215 DOI: 10.1002/pds.1630]
133 **Graveling AJ**, Deary IJ, Frier BM. Acute hypoglycemia impairs executive cognitive function in adults with and without type 1 diabetes. *Diabetes Care* 2013; **36**: 3240-3246 [PMID: 23780950 DOI: 10.2337/dc13-0194]
134 **Lacy ME**, Gilsanz P, Eng C, Beeri MS, Karter AJ, Whitmer RA. Severe Hypoglycemia and Cognitive Function in Older Adults With Type 1 Diabetes: The Study of Longevity in Diabetes (SOLID). *Diabetes Care* 2020; **43**: 541-548 [PMID: 31882410 DOI: 10.2337/dc19-0906]
135 **Weinstein G**, Davis-Plourde KL, Conner S, Himali JJ, Beiser AS, Lee A, Rawlings AM, Sedaghat S, Ding J, Moshier E, van Duijn CM, Beeri MS, Selvin E, Ikram MA, Launer LJ, Haan MN, Seshadri S. Association of metformin, sulfonylurea and insulin use with brain structure and function and risk of dementia and Alzheimer's disease: Pooled analysis from 5 cohorts. *PLoS One* 2019; **14**: e0212293 [PMID: 30768625 DOI: 10.1371/journal.pone.0212293]
136 **Verdile G**, Fuller SJ, Martins RN. The role of type 2 diabetes in neurodegeneration. *Neurobiol Dis* 2015; **84**: 22-38 [PMID: 25926349 DOI: 10.1016/j.nbd.2015.04.008]
137 **Craft S**, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. *Arch Neurol* 2012; **69**: 29-38 [PMID: 21911655 DOI: 10.1001/archneurol.2011.233]
138 **Reger MA**, Watson GS, Frey WH 2nd, Baker LD, Cholerton B, Keeling ML, Belongia DA, Fishel MA, Plymate SR, Schellenberg GD, Cherrier MM, Craft S. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. *Neurobiol Aging* 2006; **27**: 451-458 [PMID: 15964100 DOI: 10.1016/j.neurobiolaging.2005.03.016]
139 **Benedict C**, Hallschmid M, Schmitz K, Schultes B, Ratter F, Fehm HL, Born J, Kern W. Intranasal insulin improves memory in humans: superiority of insulin aspart. *Neuropsychopharmacology* 2007; **32**: 239-243 [PMID: 16936707 DOI: 10.1038/sj.npp.1301193]
140 **Reger MA**, Watson GS, Green PS, Wilkinson CW, Baker LD, Cholerton B, Fishel MA, Plymate SR, Breitner JC, DeGroodt W, Mehta P, Craft S. Intranasal insulin improves cognition and modulates beta-amyloid in early AD. *Neurology* 2008; **70**: 440-448 [PMID: 17942819 DOI: 10.1212/01.WNL.0000265401.62434.36]
141 **Craft S**, Raman R, Chow TW, Rafii MS, Rissman RA, Brewer JB, Donohue MC, Sun C-K, Harless K, Gessert D, Aisen PS. DT-02-03: Open label extension results from a phase ii/iii trial of intranasal insulin. *Alzheimers Dement* 2019; **15**: P1489-P1489 [DOI: 10.1016/j.jalz.2019.08.012]
142 **Craft S**. Study of Nasal Insulin to Fight Forgetfulness - Short-Acting Insulin Aspart (SNIFF-Quick). [accessed 2020 Dec 19]. In: ClinicalTrials.gov [Internet]. Winston-Salem (CA): U.S. National Library of Medicine. Available from: https://www.clinicaltrials.gov/ct2/show/NCT02462161 ClinicalTrials.gov Identifier: NCT02462161
143 **Rosenbloom MH**. Intranasal Glulisine in Amnestic Mild Cognitive Impairment and Probable Mild Alzheimer's Disease. [accessed 2020 Dec 16]. In: ClinicalTrials.gov [Internet]. Saint Paul (MN): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT02503501 ClinicalTrials.gov Identifier: NCT02503501
144 **de Candia P**, Matarese G. Leptin and ghrelin: Sewing metabolism onto neurodegeneration. *Neuropharmacology* 2018; **136**: 307-316 [PMID: 29248481 DOI: 10.1016/j.neuropharm.2017.12.025]
145 **McGuire MJ**, Ishii M. Leptin Dysfunction and Alzheimer's Disease: Evidence from Cellular, Animal, and Human Studies. *Cell Mol Neurobiol* 2016; **36**: 203-217 [PMID: 26993509 DOI: 10.1007/s10571-015-0282-7]
146 **Salazar J**, Chávez-Castillo M, Rojas J, Ortega A, Nava M, Pérez J, Rojas M, Espinoza C, Chacin M, Herazo Y, Angarita L, Rojas DM, D'Marco L, Bermudez V. Is "Leptin Resistance" Another Key Resistance to Manage Type 2 Diabetes? *Curr Diabetes Rev* 2020; **16**: 733-749 [PMID: 31886750 DOI: 10.2174/1573399816666191230111838]
147 **Tezapsidis N**, Johnston JM, Smith MA, Ashford JW, Casadesus G, Robakis NK, Wolozin B, Perry G, Zhu X, Greco SJ, Sarkar S. Leptin: a novel therapeutic strategy for Alzheimer's disease. *J Alzheimers Dis* 2009; **16**: 731-740 [PMID: 19387109 DOI: 10.3233/JAD-2009-1021]
148 **Frühbeck G**. Intracellular signalling pathways activated by leptin. *Biochem J* 2006; **393**: 7-20 [PMID: 16336196 DOI: 10.1042/BJ20051578]
149 **Paz-Filho G**, Mastronardi C, Wong ML, Licinio J. Leptin therapy, insulin sensitivity, and glucose homeostasis. *Indian J Endocrinol Metab* 2012; **16**: S549-S555 [PMID: 23565489 DOI: 10.4103/2230-8210.105571]

150 **German JP**, Wisse BE, Thaler JP, Oh-I S, Sarruf DA, Ogimoto K, Kaiyala KJ, Fischer JD, Matsen ME, Taborsky GJ Jr, Schwartz MW, Morton GJ. Leptin deficiency causes insulin resistance induced by uncontrolled diabetes. *Diabetes* 2010; **59**: 1626-1634 [PMID: 20424233 DOI: 10.2337/db09-1918]

151 **Harvey J**, Solovyova N, Irving A. Leptin and its role in hippocampal synaptic plasticity. *Prog Lipid Res* 2006; **45**: 369-378 [PMID: 16678906 DOI: 10.1016/j.plipres.2006.03.001]

152 **Greco SJ**, Bryan KJ, Sarkar S, Zhu X, Smith MA, Ashford JW, Johnston JM, Tezapsidis N, Casadesus G. Leptin reduces pathology and improves memory in a transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis* 2010; **19**: 1155-1167 [PMID: 20308782 DOI: 10.3233/JAD-2010-1308]

153 **Pérez-González R**, Antequera D, Vargas T, Spuch C, Bolós M, Carro E. Leptin induces proliferation of neuronal progenitors and neuroprotection in a mouse model of Alzheimer's disease. *J Alzheimers Dis* 2011; **24** Suppl 2: 17-25 [PMID: 21335656 DOI: 10.3233/JAD-2011-102070]

154 **Gómez R**, Lago F, Gómez-Reino JJ, Gualillo O. Novel factors as therapeutic targets to treat diabetes. Focus on leptin and ghrelin. *Expert Opin Ther Targets* 2009; **13**: 583-591 [PMID: 19397477 DOI: 10.1517/14728220902914834]

155 **Seminara RS**, Jeet C, Biswas S, Kanwal B, Iftikhar W, Sakibuzzaman M, Rutkofsky IH. The Neurocognitive Effects of Ghrelin-induced Signaling on the Hippocampus: A Promising Approach to Alzheimer's Disease. *Cureus* 2018; **10**: e3285 [PMID: 30443455 DOI: 10.7759/cureus.3285]

156 **Kern A**, Mavrikaki M, Ullrich C, Albarran-Zeckler R, Brantley AF, Smith RG. Hippocampal Dopamine/DRD1 Signaling Dependent on the Ghrelin Receptor. *Cell* 2015; **163**: 1176-1190 [PMID: 26590421 DOI: 10.1016/j.cell.2015.10.062]

157 **Tian J**, Guo L, Sui S, Driskill C, Phensy A, Wang Q, Gauba E, Zigman JM, Swerdlow RH, Kroener S, Du H. Disrupted hippocampal growth hormone secretagogue receptor 1α interaction with dopamine receptor D1 plays a role in Alzheimer's disease. *Sci Transl Med* 2019; **11** [PMID: 31413143 DOI: 10.1126/scitranslmed.aav6278]

158 **Kunath N**, van Groen T, Allison DB, Kumar A, Dozier-Sharpe M, Kadish I. Ghrelin agonist does not foster insulin resistance but improves cognition in an Alzheimer's disease mouse model. *Sci Rep* 2015; **5**: 11452 [PMID: 26090621 DOI: 10.1038/srep11452]

159 **Chen Y**, Cao CP, Li CR, Wang W, Zhang D, Han LL, Zhang XQ, Kim A, Kim S, Liu GL. Ghrelin modulates insulin sensitivity and tau phosphorylation in high glucose-induced hippocampal neurons. *Biol Pharm Bull* 2010; **33**: 1165-1169 [PMID: 20606308 DOI: 10.1248/bpb.33.1165]

160 **Dhurandhar EJ**, Allison DB, van Groen T, Kadish I. Hunger in the absence of caloric restriction improves cognition and attenuates Alzheimer's disease pathology in a mouse model. *PLoS One* 2013; **8**: e60437 [PMID: 23565247 DOI: 10.1371/journal.pone.0060437]

161 **Eslami M**, Sadeghi B, Goshadrou F. Chronic ghrelin administration restores hippocampal long-term potentiation and ameliorates memory impairment in rat model of Alzheimer's disease. *Hippocampus* 2018; **28**: 724-734 [PMID: 30009391 DOI: 10.1002/hipo.23002]

162 **Santos VV**, Stark R, Rial D, Silva HB, Bayliss JA, Lemus MB, Davies JS, Cunha RA, Prediger RD, Andrews ZB. Acyl ghrelin improves cognition, synaptic plasticity deficits and neuroinflammation following amyloid β (Aβ1-40) administration in mice. *J Neuroendocrinol* 2017; **29** [PMID: 28380673 DOI: 10.1111/jne.12476]

163 **Hsu CC**, Wahlqvist ML, Lee MS, Tsai HN. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J Alzheimers Dis* 2011; **24**: 485-493 [PMID: 21297276 DOI: 10.3233/JAD-2011-101524]

164 **Alp H**, Varol S, Celik MM, Altas M, Evliyaoglu O, Tokgoz O, Tanrıverdi MH, Uzar E. Protective effects of beta glucan and gliclazide on brain tissue and sciatic nerve of diabetic rats induced by streptozosin. *Exp Diabetes Res* 2012; **2012**: 230342 [PMID: 22291696 DOI: 10.1155/2012/230342]

165 **Baraka A**, ElGhotny S. Study of the effect of inhibiting galanin in Alzheimer's disease induced in rats. *Eur J Pharmacol* 2010; **641**: 123-127 [PMID: 20639139 DOI: 10.1016/j.ejphar.2010.05.030]

166 **Abdallah DM**, Nassar NN, Abd-El-Salam RM. Glibenclamide ameliorates ischemia-reperfusion injury *via* modulating oxidative stress and inflammatory mediators in the rat hippocampus. *Brain Res* 2011; **1385**: 257-262 [PMID: 21316351 DOI: 10.1016/j.brainres.2011.02.007]

167 **Schloot NC**, Haupt A, Schütt M, Badenhoop K, Laimer M, Nicolay C, Reaney M, Fink K, Holl RW. Risk of severe hypoglycemia in sulfonylurea-treated patients from diabetes centers in Germany/Austria: How big is the problem? *Diabetes Metab Res Rev* 2016; **32**: 316-324 [PMID: 26409039 DOI: 10.1002/dmrr.2722]

168 **Schopman JE**, Simon AC, Hoefnagel SJ, Hoekstra JB, Scholten RJ, Holleman F. The incidence of mild and severe hypoglycaemia in patients with type 2 diabetes mellitus treated with sulfonylureas: a systematic review and meta-analysis. *Diabetes Metab Res Rev* 2014; **30**: 11-22 [PMID: 24030920 DOI: 10.1002/dmrr.2470]

169 **Kickstein E**, Krauss S, Thornhill P, Rutschow D, Zeller R, Sharkey J, Williamson R, Fuchs M, Köhler A, Glossmann H, Schneider R, Sutherland C, Schweiger S. Biguanide metformin acts on tau phosphorylation *via* mTOR/protein phosphatase 2A (PP2A) signaling. *Proc Natl Acad Sci USA* 2010; **107**: 21830-21835 [PMID: 21098287 DOI: 10.1073/pnas.0912793107]

170 **Li SN**, Wang X, Zeng QT, Feng YB, Cheng X, Mao XB, Wang TH, Deng HP. Metformin inhibits nuclear factor kappaB activation and decreases serum high-sensitivity C-reactive protein level in experimental atherogenesis of rabbits. *Heart Vessels* 2009; **24**: 446-453 [PMID: 20108078 DOI: 10.1007/s00380-008-1137-7]

171 **Rena G**, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: old or new insights? *Diabetologia* 2013; **56**: 1898-1906 [PMID: 23835523 DOI: 10.1007/s00125-013-2991-0]

172 **Burcelin R**. The antidiabetic gutsy role of metformin uncovered? *Gut* 2014; **63**: 706-707 [PMID: 23840042 DOI: 10.1136/gutjnl-2013-305370]

173 **Ibrahim OHM**, Hassan MA. The Use of Anti-Diabetic Drugs in Alzheimer's Disease, New Therapeutic Options and Future Perspective. *Pharmacol Amp Pharm* 2018; **9**: 157-174 [DOI: 10.4236/pp.2018.96013]

174 **Mostafa DK**, Ismail CA, Ghareeb DA. Differential metformin dose-dependent effects on cognition in rats: role of Akt. *Psychopharmacology (Berl)* 2016; **233**: 2513-2524 [PMID: 27113224 DOI: 10.1007/s00213-016-4301-2]

175 **Imfeld P**, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. *J Am Geriatr Soc* 2012; **60**: 916-921 [PMID: 22458300 DOI: 10.1111/j.1532-5415.2012.03916.x]

176 **Wang L**, Liu W, Fan Y, Liu T, Yu C. Effect of rosiglitazone on amyloid precursor protein processing and Aβ clearance in streptozotocin-induced rat model of Alzheimer's disease. *Iran J Basic Med Sci* 2017; **20**: 474-480 [PMID: 28656081 DOI: 10.22038/IJBMS.2017.8669]

177 **Pancani T**, Phelps JT, Searcy JL, Kilgore MW, Chen KC, Porter NM, Thibault O. Distinct modulation of voltage-gated and ligand-gated Ca2+ currents by PPAR-gamma agonists in cultured hippocampal neurons. *J Neurochem* 2009; **109**: 1800-1811 [PMID: 19453298 DOI: 10.1111/j.1471-4159.2009.06107.x]

178 **Femminella GD**, Bencivenga L, Petraglia L, Visaggi L, Gioia L, Grieco FV, de Lucia C, Komici K, Corbi G, Edison P, Rengo G, Ferrara N. Antidiabetic Drugs in Alzheimer's Disease: Mechanisms of Action and Future Perspectives. *J Diabetes Res* 2017; **2017**: 7420796 [PMID: 28656154 DOI: 10.1155/2017/7420796]

179 **Cheng H**, Shang Y, Jiang L, Shi TL, Wang L. The peroxisome proliferators activated receptor-gamma agonists as therapeutics for the treatment of Alzheimer's disease and mild-to-moderate Alzheimer's disease: a meta-analysis. *Int J Neurosci* 2016; **126**: 299-307 [PMID: 26001206 DOI: 10.3109/00207454.2015.1015722]

180 **Sato T**, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer disease. *Neurobiol Aging* 2011; **32**: 1626-1633 [PMID: 19923038 DOI: 10.1016/j.neurobiolaging.2009.10.009]

181 **Geldmacher DS**, Fritsch T, McClendon MJ, Landreth G. A randomized pilot clinical trial of the safety of pioglitazone in treatment of patients with Alzheimer disease. *Arch Neurol* 2011; **68**: 45-50 [PMID: 20837824 DOI: 10.1001/archneurol.2010.229]

182 **Takeda**. Biomarker Qualification for Risk of Mild Cognitive Impairment (MCI) Due to Alzheimer's Disease (AD) and Safety and Efficacy Evaluation of Pioglitazone in Delaying Its Onset (TOMMORROW). [accessed 2020 Dec 20]. In: ClinicalTrials.gov [Internet]. Zinfandel Pharmaceuticals Inc.: U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT01931566 ClinicalTrials.gov Identifier: NCT01931566

183 **Hölscher C**. Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. *J Endocrinol* 2014; **221**: T31-T41 [PMID: 23999914 DOI: 10.1530/JOE-13-0221]

184 **McClean PL**, Parthsarathy V, Faivre E, Hölscher C. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *J Neurosci* 2011; **31**: 6587-6594 [PMID: 21525299 DOI: 10.1523/JNEUROSCI.0529-11.2011]

185 **Han WN**, Hölscher C, Yuan L, Yang W, Wang XH, Wu MN, Qi JS. Liraglutide protects against amyloid-β protein-induced impairment of spatial learning and memory in rats. *Neurobiol Aging* 2013; **34**: 576-588 [PMID: 22592020 DOI: 10.1016/j.neurobiolaging.2012.04.009]

186 **Parthsarathy V**, Hölscher C. Chronic treatment with the GLP1 analogue liraglutide increases cell proliferation and differentiation into neurons in an AD mouse model. *PLoS One* 2013; **8**: e58784 [PMID: 23536825 DOI: 10.1371/journal.pone.0058784]

187 **Kelly P**, McClean PL, Ackermann M, Konerding MA, Hölscher C, Mitchell CA. Restoration of cerebral and systemic microvascular architecture in APP/PS1 transgenic mice following treatment with Liraglutide™. *Microcirculation* 2015; **22**: 133-145 [PMID: 25556713 DOI: 10.1111/micc.12186]

188 **Qi L**, Ke L, Liu X, Liao L, Ke S, Wang Y, Lin X, Zhou Y, Wu L, Chen Z, Liu L. Subcutaneous administration of liraglutide ameliorates learning and memory impairment by modulating tau hyperphosphorylation *via* the glycogen synthase kinase-3β pathway in an amyloid β protein induced alzheimer disease mouse model. *Eur J Pharmacol* 2016; **783**: 23-32 [PMID: 27131827 DOI: 10.1016/j.ejphar.2016.04.052]

189 **Gejl M**, Gjedde A, Egefjord L, Møller A, Hansen SB, Vang K, Rodell A, Brændgaard H, Gottrup H, Schacht A, Møller N, Brock B, Rungby J. In Alzheimer's Disease, 6-Month Treatment with GLP-1 Analog Prevents Decline of Brain Glucose Metabolism: Randomized, Placebo-Controlled, Double-Blind Clinical Trial. *Front Aging Neurosci* 2016; **8**: 108 [PMID: 27252647 DOI: 10.3389/fnagi.2016.00108]

190 **Edison P**. Evaluating Liraglutide in Alzheimer's Disease (ELAD). [accessed 2020 Dec 19]. In: ClinicalTrials.gov [Internet]. London: U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT01843075 ClinicalTrials.gov Identifier: NCT01843075

191 **Gault VA**, Hölscher C. Protease-resistant glucose-dependent insulinotropic polypeptide agonists facilitate hippocampal LTP and reverse the impairment of LTP induced by beta-amyloid. *J*

Neurophysiol 2008; **99**: 1590-1595 [PMID: 18234983 DOI: 10.1152/jn.01161.2007]

192 **Duffy AM**, Hölscher C. The incretin analogue D-Ala2GIP reduces plaque load, astrogliosis and oxidative stress in an APP/PS1 mouse model of Alzheimer's disease. *Neuroscience* 2013; **228**: 294-300 [PMID: 23103794 DOI: 10.1016/j.neuroscience.2012.10.045]

193 **Faivre E**, Hölscher C. Neuroprotective effects of D-Ala(2)GIP on Alzheimer's disease biomarkers in an APP/PS1 mouse model. *Alzheimers Res Ther* 2013; **5**: 20 [PMID: 23601582 DOI: 10.1186/alzrt174]

194 **Hölscher C**. Novel dual GLP-1/GIP receptor agonists show neuroprotective effects in Alzheimer's and Parkinson's disease models. *Neuropharmacology* 2018; **136**: 251-259 [PMID: 29402504 DOI: 10.1016/j.neuropharm.2018.01.040]

195 **Li T**, Jiao JJ, Hölscher C, Wu MN, Zhang J, Tong JQ, Dong XF, Qu XS, Cao Y, Cai HY, Su Q, Qi JS. A novel GLP-1/GIP/Gcg triagonist reduces cognitive deficits and pathology in the 3xTg mouse model of Alzheimer's disease. *Hippocampus* 2018; **28**: 358-372 [PMID: 29473979 DOI: 10.1002/hipo.22837]

196 **Camins A**, Ettcheto M, Busquets O, Manzine PR, Castro-Torres RD, Beas-Zarate C, Verdaguer E, Sureda FX, Bulló M, Olloquequi J, Auladell C, Folch J. Triple GLP-1/GIP/glucagon receptor agonists, a potential novel treatment strategy in Alzheimer's disease. *Expert Opin Investig Drugs* 2019; **28**: 93-97 [PMID: 30480461 DOI: 10.1080/13543784.2019.1552677]

197 **Kosaraju J**, Gali CC, Khatwal RB, Dubala A, Chinni S, Holsinger RM, Madhunapantula VS, Muthureddy Nataraj SK, Basavan D. Saxagliptin: a dipeptidyl peptidase-4 inhibitor ameliorates streptozotocin induced Alzheimer's disease. *Neuropharmacology* 2013; **72**: 291-300 [PMID: 23603201 DOI: 10.1016/j.neuropharm.2013.04.008]

198 **Kosaraju J**, Murthy V, Khatwal RB, Dubala A, Chinni S, Muthureddy Nataraj SK, Basavan D. Vildagliptin: an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. *J Pharm Pharmacol* 2013; **65**: 1773-1784 [PMID: 24117480 DOI: 10.1111/jphp.12148]

199 **Wiciński M**, Wódkiewicz E, Górski K, Walczak M, Malinowski B. Perspective of SGLT2 Inhibition in Treatment of Conditions Connected to Neuronal Loss: Focus on Alzheimer's Disease and Ischemia-Related Brain Injury. *Pharmaceuticals (Basel)* 2020; **13** [PMID: 33187206 DOI: 10.3390/ph13110379]

200 **Sa-Nguanmoo P**, Tanajak P, Kerdphoo S, Jaiwongkam T, Pratchayasakul W, Chattipakorn N, Chattipakorn SC. SGLT2-inhibitor and DPP-4 inhibitor improve brain function *via* attenuating mitochondrial dysfunction, insulin resistance, inflammation, and apoptosis in HFD-induced obese rats. *Toxicol Appl Pharmacol* 2017; **333**: 43-50 [PMID: 28807765 DOI: 10.1016/j.taap.2017.08.005]

201 **Millar P**, Pathak N, Parthsarathy V, Bjourson AJ, O'Kane M, Pathak V, Moffett RC, Flatt PR, Gault VA. Metabolic and neuroprotective effects of dapagliflozin and liraglutide in diabetic mice. *J Endocrinol* 2017; **234**: 255-267 [PMID: 28611211 DOI: 10.1530/JOE-17-0263]

202 **Lin B**, Koibuchi N, Hasegawa Y, Sueta D, Toyama K, Uekawa K, Ma M, Nakagawa T, Kusaka H, Kim-Mitsuyama S. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol* 2014; **13**: 148 [PMID: 25344694 DOI: 10.1186/s12933-014-0148-1]

203 **Hierro-Bujalance C**, Infante-Garcia C, Del Marco A, Herrera M, Carranza-Naval MJ, Suarez J, Alves-Martinez P, Lubian-Lopez S, Garcia-Alloza M. Empagliflozin reduces vascular damage and cognitive impairment in a mixed murine model of Alzheimer's disease and type 2 diabetes. *Alzheimers Res Ther* 2020; **12**: 40 [PMID: 32264944 DOI: 10.1186/s13195-020-00607-4]

204 **Wium-Andersen IK**, Osler M, Jørgensen MB, Rungby J, Wium-Andersen MK. Antidiabetic medication and risk of dementia in patients with type 2 diabetes: a nested case-control study. *Eur J Endocrinol* 2019; **181**: 499-507 [PMID: 31437816 DOI: 10.1530/EJE-19-0259]

205 **Burns J**. Dapagliflozin In Alzheimer's Disease. [accessed 2021 Mar 14]. In: ClinicalTrials.gov [Internet]. Kansas City (KS): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT03801642 ClinicalTrials.gov Identifier: NCT03801642

206 **Dhananjayan K**, Forbes J, Münch G. Advanced Glycation, Diabetes, and Dementia. In: Srikanth V, Arvanitakis Z. Type 2 Diabetes and Dementia. Elsevier, 2018: 169-193

207 **Burstein AH**, Sabbagh M, Andrews R, Valcarce C, Dunn I, Altstiel L. Development of Azeliragon, an Oral Small Molecule Antagonist of the Receptor for Advanced Glycation Endproducts, for the Potential Slowing of Loss of Cognition in Mild Alzheimer's Disease. *J Prev Alzheimers Dis* 2018; **5**: 149-154 [PMID: 29616709 DOI: 10.14283/jpad.2018.18]

208 **vTv Therapeutics**. Study of Azeliragon in Patients With Mild Alzheimer's Disease and Impaired Glucose Tolerance (Elevage). [accessed 2020 Dec 19]. In: ClinicalTrials.gov [Internet]. Tucson (AZ): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT03980730 ClinicalTrials.gov Identifier: NCT03980730

209 **Adler BL**, Yarchoan M, Hwang HM, Louneva N, Blair JA, Palm R, Smith MA, Lee HG, Arnold SE, Casadesus G. Neuroprotective effects of the amylin analogue pramlintide on Alzheimer's disease pathogenesis and cognition. *Neurobiol Aging* 2014; **35**: 793-801 [PMID: 24239383 DOI: 10.1016/j.neurobiolaging.2013.10.076]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 767-779

DOI: 10.4239/wjd.v12.i6.767

ISSN 1948-9358 (online)

MINIREVIEWS

Diabetes and inflammatory diseases: An overview from the perspective of Ca^{2+}/3'-5'-cyclic adenosine monophosphate signaling

Leandro Bueno Bergantin

ORCID number: Leandro Bueno Bergantin 0000-0001-7432-9958.

Author contributions: Bergantin LB is the sole author; Bergantin L participated in the whole process of the study.

Conflict-of-interest statement: The author declares no conflict of interest, financial or otherwise.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Brazil

Peer-review report's scientific quality classification

Leandro Bueno Bergantin, Department ofPharmacology, Universidade Federal de São Paulo, São Paulo 04039-032, Brazil

Corresponding author: Leandro Bueno Bergantin, PhD, Professor, Department of Pharmacology, Universidade Federal de São Paulo, Rua Pedro de Toledo, 669–Vila Clementino, São Paulo 04039-032, Brazil. leanbio39@yahoo.com.br

Abstract

A large amount of evidence has supported a clinical link between diabetes and inflammatory diseases, *e.g.*, cancer, dementia, and hypertension. In addition, it is also suggested that dysregulations related to Ca^{2+} signaling could link these diseases, in addition to 3'-5'-cyclic adenosine monophosphate (cAMP) signaling pathways. Thus, revealing this interplay between diabetes and inflammatory diseases may provide novel insights into the pathogenesis of these diseases. Publications involving signaling pathways related to Ca^{2+} and cAMP, inflammation, diabetes, dementia, cancer, and hypertension (alone or combined) were collected by searching PubMed and EMBASE. Both signaling pathways, Ca^{2+} and cAMP signaling, control the release of neurotransmitters and hormones, in addition to neurodegeneration, and tumor growth. Furthermore, there is a clear relationship between Ca^{2+} signaling, *e.g.*, increased Ca^{2+} signals, and inflammatory responses. cAMP also regulates pro- and anti-inflammatory responses. Due to the experience of our group in this field, this article discusses the role of Ca^{2+} and cAMP signaling in the correlation between diabetes and inflammatory diseases, including its pharmacological implications. As a novelty, this article also includes: (1) A timeline of the major events in Ca^{2+}/cAMP signaling; and (2) As coronavirus disease 2019 (COVID-19) is an emerging and rapidly evolving situation, this article also discusses recent reports on the role of Ca^{2+} channel blockers for preventing Ca^{2+} signaling disruption due to COVID-19, including the correlation between COVID-19 and diabetes.

Key Words: Diabetes; Cancer; Hypertension; Dementia; Ca^{2+}/3'-5'-cyclic adenosine monophosphate signaling; Ca^{2+} channel blockers; Pharmacotherapy; Neurodegeneration; COVID-19

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

Received: December 2, 2020
Peer-review started: December 2, 2020
First decision: December 24, 2020
Revised: December 29, 2020
Accepted: March 7, 2021
Article in press: March 7, 2021
Published online: June 15, 2021

P-Reviewer: Aureliano M, Avtanski D
S-Editor: Fan JR
L-Editor: Webster JR
P-Editor: Yuan YY

Core Tip: There are several reviews in the literature on diabetes and inflammatory diseases. Nonetheless, to my knowledge, this is the first review which clearly discusses the role of Ca^{2+}/3'-5'-cyclic adenosine monophosphate (cAMP) signaling in the link between diabetes and inflammatory diseases. This article also includes a timeline of the major events in Ca^{2+}/cAMP signaling, and discusses recent reports on the role of Ca^{2+} channel blockers for preventing Ca^{2+} signaling disruption due to coronavirus disease 2019 (COVID-19), including the correlation between COVID-19 and diabetes.

Citation: Bergantin LB. Diabetes and inflammatory diseases: An overview from the perspective of Ca^{2+}/3'-5'-cyclic adenosine monophosphate signaling. *World J Diabetes* 2021; 12(6): 767-779
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/767.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.767

INTRODUCTION

The concept of a complex clinical link between diabetes and inflammatory diseases, *e.g.*, cancer, dementia, and hypertension, has been described in several reports[1-12]. Observational reports provided the first evidence for the link between inflammation and diabetes[11]. An interesting study correlated inflammation and diabetes by showing, in animal models, that tumor necrosis factor-alpha is correlated with both obesity and insulin resistance[13]. In addition, an epidemiologic link between inflammation and diabetes was made when circulating concentrations of markers, and mediators, of inflammation were demonstrated to be increased in these diseases[14-17], *e.g.*, white cells, interleukin (IL)-6, fibrinogen, C-reactive protein, plasminogen activator inhibitor-1, and sialic acid. Besides diabetes, the link between cancer and inflammation was proposed in 1863 by Virchow, which postulated that the etiology of cancer is related to chronic inflammation[18]. It is now clear that persistent cell proliferation at sites that are abundant in inflammatory cells, DNA-damage-promoting agents, growth factors, and activated stroma, actually increases the risk of neoplasia [19]. Inflammation also plays a central role in dementia, *e.g.*, Alzheimer's disease (AD) [20]. For instance, persistent stimulation of immune cells, and macrophages in the brain (microglia), has been shown to aggravate both amyloid and tau pathology, which then may operate as a link in the etiology of the disease[20]. Finally, recent interest has focused on studying the avenues, and inflammatory mediators, through which immune cells operate to lead to hypertension and end-organ injury[21].

Currently, it is also well-known that dyshomeostasis of Ca^{2+}, through an increase in Ca^{2+} levels within the cells $[Ca^{2+}]c$, is correlated with the pathogenesis of diabetes, cancer, hypertension, and dementia[9,10,12,22-26]. In fact, there is a clear relationship between Ca^{2+} signaling, *e.g.*, increased Ca^{2+} signals, and inflammatory responses[10,27]. Corroborating this idea, several studies have highlighted that Ca^{2+} channel blockers (CCBs), classic antihypertensive medicines, can improve cognitive function, in addition to decreasing the symptoms of both cancer and diabetes[12,28-31]. A pharmacological tenet for these exciting findings is linked with reestablishing $[Ca^{2+}]c$, in addition to modulating 3'-5'-cyclic adenosine monophosphate (cAMP) signaling pathways (Ca^{2+}/cAMP signaling)[9,10,24,25]. Due to the experience of our group in this field[9,10,12,24,25,32-36], this article discusses the contributions of Ca^{2+} and cAMP signaling in the correlation between cancer, hypertension, diabetes, and dementia. Publications involving dementia, diabetes, hypertension, and cancer were obtained by examining PubMed and EMBASE, using a search strategy with a high sensitivity for studies of etiology, as follows: (1) Searches applied the following strings: Risk (in title or abstract) odds ratio (OR) risk [as a Medical Subject Heading (MeSH) term, not exploded] OR cohort studies (as a MeSH term) OR group (as a text word). Outcomes of these searches were linked with sets conceived with diabetes OR dementia OR cancer OR hypertension OR inflammation; and (2) Bibliographies of the articles obtained were also reviewed for possible data sources.

This article included as a novelty: (1) A timeline of the major events in Ca^{2+}/cAMP signaling; and (2) As coronavirus disease 2019 (COVID-19) is an emerging and rapidly evolving situation, this article also discusses recent reports on the role of CCBs for preventing Ca^{2+} signaling disruption due to COVID-19, including the correlation

between COVID-19 and diabetes.

CANCER, HYPERTENSION, DIABETES, AND DEMENTIA: A CLINICAL LINK

Basic mechanisms

Dementia: Dementia, like AD, and aging are classically associated with each other[10,24,37,38]. Dementia is often characterized by synaptic dysfunction and death of neurons, leading to a gradual decline in cognitive abilities[24,38]. Among the hallmark features of AD, an accumulation of plaques containing the amyloid beta (Aβ) peptide is established; then in the preclinical phase of AD, Aβ is among the most prevalent pathological markers[10,24,38]. Briefly, the amyloid precursor protein (APP) undergoes proteolysis, thus producing Aβ as a product, which can be quantified from both blood and cerebrospinal fluid, and by imaging[10,24]. In addition, Ca^{2+} dyshomeostasis has also been associated with dementia, resulting in the death of neurons[10,24,33,37]. Aβ has been linked with both an increased $[Ca^{2+}]_c$ and an enhanced susceptibility to neuroexcitotoxicity[10,37]. Ca^{2+} signaling has been investigated due to modulation of neuronal death[37].

Cancer: Like dementia, cancer dramatically affects the health of individuals, and is considered an uncontrolled division of the body's cells[9,39]. Ca^{2+} dysregulations have also been observed in carcinogenesis[9,39-44]. For example, several studies corroborated the involvement of Ca^{2+} channels overexpression, or hyperactivation, in different types of cancer[40-44]. For instance, a Ca^{2+}-binding protein that exerts a protagonist role in intracellular Ca^{2+} homeostasis is regucalcin (RGN), a protein which was observed to have reduced expression in cancer of the prostate gland[45,46]. It has also been observed that an elevation of $[Ca^{2+}]c$ stimulates diverse responses to the proliferation of cells from both neoplastic and non-neoplastic cancer of the prostate gland, suggesting a correlation with RGN expression[9,45,46]. Furthermore, increased expression of RGN reduced the migration of NSCLC A549 cells from adenocarcinoma of lung *in vitro*[45,46]. The protein and mRNA expression of RGN was also decreased in (1) HepG2 cells from human hepatoma; (2) MCF-7 cells from breast cancer; and (3) LNCaP cells from prostate cancer[35,36]. Thus, reduced expression of RGN may be correlated with the stimulation of carcinogenesis[45,46]. Besides RGN, there are other Ca^{2+}-binding proteins that regulate Ca^{2+} homeostasis, which are implicated in cancer. In addition, expression levels of S100B, TM4SF3 and OLFM4 have been discovered to be highly associated with metastasis of liver cancer[9,47]. These findings confirm the participation of Ca^{2+} dyshomeostasis in cancer, opening new perspectives for the advancement of therapeutics linked to Ca^{2+} signaling.

Hypertension: Sympathetic hyperactivity, due to dysregulation of Ca^{2+} signaling as in dementia and cancer, has been correlated with hypertension. In fact, studies by Miranda-Ferreira *et al*[48,49] validated this principle by demonstrating changes in the kinetics of the release of catecholamines from spontaneously hypertensive rats (SHRs), when compared with normotensive rats. Ca^{2+} signaling is argued to be an issue involved in these differences. The authors[48,49] reinforced that Ca^{2+} dyshomeostasis might explain the increased release of catecholamines seen in SHRs, when compared with normotensive rats.

Diabetes: Like dementia, cancer, and hypertension, diabetes is also a serious medical condition. Diabetes is presently categorized according to its origin. For example, in type 1 diabetes if there is a deficiency of insulin released by the pancreas, then it can be categorized as a juvenile diabetes; while in type 2 diabetes if there is resistance to insulin, then it can be categorized as adult-onset diabetes[50]. From a cellular point of view, whereas a physiological increase in the cytoplasmic concentration of Ca^{2+} is a significant trigger for releasing insulin, an abnormal elevation of $[Ca^{2+}]c$ could stimulate β-cell apoptosis, then decrease insulin levels, contributing to diabetes[9,12,25]. Besides Ca^{2+}, cAMP modulates the release of various hormones, including insulin released from pancreatic β-cells[51,52]. Although increasing cAMP levels, *e.g.*, *via* adrenaline, might stimulate the production of hepatic glucose, increasing levels of cAMP in pancreatic β-cells may stimulate insulin release. The start signal for release of insulin is achieved by elevating $[Ca^{2+}]c$, and this signal is later amplified *via* cAMP[53]. Additionally, cAMP is also implicated in other cellular phenomena of β-cells, *e.g.*, inhibiting apoptosis[53].

Nowadays, a clinical link between these discussed diseases (hypertension, cancer, diabetes, and dementia) has been described in several reports[1-12,25,26].

A clinical link

Cancer and dementia: A link between dementia and cancer can be established through numerous cellular phenomena that are implicated in the etiology of both diseases, *e.g.*, inflammation, oxidative stress, and angiogenesis[1,3,54]. For instance, inflammatory biomarkers linked with a lower cognitive performance have been shown to be increased, including fibrinogen and IL-6[1-3]. In fact, several proteins may be involved in the etiology of this link, *e.g.*, Aβ peptide[1-3]. It is suggested that Aβ peptide overexpression is correlated with cancer as the overexpression of APP has been found in several tumors, and was then linked with cell proliferation, migration, and invasion [1]. In addition, a recognized tumor suppressor protein, the BRCA1 protein, has been linked to AD[2]. Thus, Aβ pathology can partially result from overactivation of BRCA1, and then promote neurodegeneration[2]. Converging with this concept, plasma levels of Aβ peptide have been found to be increased in patients with different cancer types[3].

In addition, a deficiency in DNA repair mechanisms and/or oxidative stress could lead to DNA damage, an issue which is also important for the etiology of both cancer and dementia[55]. A reduced capacity to repair damaged DNA due to genetic polymorphisms can be correlated to an augmented risk of both cancer and cognitive impairment. Genetic defects in DNA damage repair mechanisms could lead to syndromes such as xeroderma pigmentosum and ataxia telangiectasia, characterized by an augmented risk of cancer and cognitive problems[55]. Thus, understanding the clinical link between cancer and dementia could result in novel therapeutics for both diseases. Therefore, it is essential to determine the etiology of this link, *e.g.*, by analyzing the preclinical phases of both diseases.

Hypertension and cancer: A correlation between hypertension and a higher incidence of cancer has been established by epidemiological and clinical reports[4-8]. However, this correlation is not completely elucidated, and has been highly discussed. For example, the Metabolic Syndrome and Cancer Project assessed this issue, and included cohort studies from Norway, Austria, and Sweden[4]. The goal of the Metabolic Syndrome and Cancer Project was to study the association between metabolic issues and the increased incidence of cancer[4,5]. Patients from cohorts related to the Metabolic Syndrome and Cancer Project were enrolled in health inspections between the 1970's and 2000's[4]. The study observed a strong correlation between hypertension and an enhanced incidence of prostate, oropharynx, rectum, pancreas, bladder, lung, and kidney cancer[4]. Additionally, strong correlations between hypertension and an enhanced incidence of pancreas, breast, corpus uteri cancer and malignant melanoma were observed in women. A positive correlation was also observed for esophagus cancer in men and women[4,5]. Indeed, cancer incidence is augmented linearly by increasing blood pressure levels[4-6]. The augmentation of cancer incidence among men was 1% to 2% points higher in hypertensive patients, compared with normotensive men[4-6].

Finally, in observational reports on renal cell carcinoma, hypertension has been documented as a cancer risk factor[4-8]. A meta-analysis of 18 studies observed a 1.6-fold increase in the incidence of renal cell carcinoma in hypertensive patients[7]. Nonetheless, this positive association between hypertension and increased cancer incidence could occur in other disorders, such as obesity[7,8]. Moreover, CCBs, antihypertensive drugs which decrease the influx of Ca^{2+} into the cells, have shown anti-cancer activity[31].

Diabetes and hypertension: Diabetes is correlated with a higher risk of developing hypertension[56]. Several findings reinforce a clear interaction between diabetes and hypertension[57]. Scientific data suggest that obesity, inflammation, oxidative stress, and insulin resistance could be associated with these diseases[56,57]. Advances in the knowledge of how to prevent these diseases may provide new insights, and perspectives, for the treatment of both diseases.

In fact, hypertension and diabetes are highlighted as the leading risk factors for atherosclerosis, including heart attacks and strokes[58]. For instance, in the Hong Kong Cardiovascular Risk Factor Prevalence report, just 42% of patients with diabetes had regular blood pressure, and just 56% of patients with hypertension had regular glucose homeostasis[58]. In the United States, patients with type 2 diabetes have a prevalence of hypertension ranging from approximately 50% to 80%[59]. In fact, a prospective cohort report from the United States concluded that hypertensive patients

had an increased risk of almost 2.5-fold for developing type 2 diabetes[60]. It is clear that diabetes and hypertension are present in the same individual more often than would occur by causality, suggesting both common genetic and environmental factors in their etiology.

Diabetes and dementia: The concept of an association between diabetes and memory dysfunctions has been frequently explored[50,61-63]. Type 2 diabetes has been linked with a decrease in both processing and speed of psychomotor functioning, in addition to a memory deficit associated with speech and fluency[61-63]. Additionally, patients with diabetes have a lengthier walking pace[61-63]. In fact, mild cognitive impairment was observed in approximately 42% of diabetic patients[64]. The association between diabetes and cognitive imbalance was examined in a report[65], and the authors concluded that patients with type 2 diabetes had a lower score in the Mini-Mental State Examination[65]. An interesting study[66] assessed if lesions in the brain, associated with both vascular and degenerative disorders, could be the cause of the association between diabetes and cognitive deficit[66]. The authors concluded that memory performance in diabetic patients was significantly reduced[66].

In addition, these findings were confirmed by reports involving neuroimaging[67]. Brain atrophy was concluded to be highly correlated with type 2 diabetes[67], which usually progresses up to 3 times faster[68,69]. Patients suffering from type 2 diabetes have also demonstrated an enhanced incidence of dementia, *e.g.*, AD[70,71]. In fact, approximately 17.5% of people with type 2 diabetes have shown a modest to a serious deficiency in day-to-day activities[72,73], while 11.3% have shown a loss of cognition, and 14.2% have shown symptoms of depression[74], consequently adversely influencing cognition[75].

A decrease in brain glucose metabolism manifested before the beginning of a quantifiable cognitive decline in cohorts of patients at risk of AD[76-79]. Reports from *in vitro* and animal experiments propose that a decrease in brain glucose metabolism comes first and, therefore, may stimulate the neuropathologic cascade, finally resulting in cognitive decline in AD[76,77]. Additionally, aging is associated with an augmented risk of worsening glucose homeostasis, which, in turn, may increase the risk of decreasing brain glucose uptake[76,79]. Thus, pharmacotherapy to decrease the risk of AD could provide the following: (1) Improve insulin sensitivity, and then restore glucose homeostasis; or (2) Reduce the decline in brain glucose metabolism by applying strategies that carefully stimulate a maintainable ketonemia[76,79].

Diabetes and cancer: A relationship between diabetes and an increased risk of several types of cancer has also been described[9,18,19]. It is now clear that persistent cell proliferation in conditions with abundant inflammatory cells, growth factors, stimulated stroma, and DNA-damage-promoting molecules, increases neoplastic risk [19]. In addition, an inflammatory process is also involved in the etiology of diabetes [17], thus it could be an issue which could link both diseases.

In fact, in laboratory reports, metformin, the most frequently used drug in patients with type 2 diabetes, has been demonstrated to prevent cell proliferation and decrease colony development, causing partial cell cycle arrest in cancer cell phenotypes[9,19]; thus, it is important not to neglect the disruption of glucose homeostasis as a relevant mediator of this link between diabetes and cancer.

Hypertension and dementia: Midlife hypertension (aged 40-64 years) increases the incidence of AD in later life (≥ 65 years); and hypertension has been linked with increased amyloid deposition and neurofibrillary tangles, both hallmarks of AD[12]. Indeed, the brain of patients with hypertension, when compared with normotensive patients, had higher concentrations of β-amyloid plaques, atrophy, and neurofibrillary tangles[12,20]. Thus, hypertension has been recognized as a risk factor for the deposition of cortical fibrillar β-amyloid[12].

Diabetes and COVID-19: COVID-19, triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an emerging and rapidly evolving situation[80,81]. It is accepted that angiotensin-converting enzyme 2, which is a component of the renin-angiotensin-aldosterone system, is the key entry receptor for SARS-CoV-2[80,81].

Intriguingly, some reports have observed increased severity of COVID-19 in patients with diabetes. To assess this issue, a meta-analysis[80] was performed by conducting a literature review of Scopus, PubMed, Science Direct, and Web of Science. Observational studies, case-reports, and case-series reports that analyzed diabetes in COVID-19 patients were included in the meta-analysis[80]. It was concluded that diabetes is a risk factor, and plays a role in disease severity and the mortality of individuals with COVID-19. This study also provided suggestions, and guidelines,

which could be helpful for the prevention and treatment of diabetic patients suffering from COVID-19[80]. As already discussed in the present article, hyperglycemia may modulate both immune and inflammatory processes, thus prejudicing patients and resulting in severe COVID-19, and possible fatal consequences.

In addition, a retrospective clinical report involving hospitalized patients with COVID-19 and hypertension, concluded that therapy with the CCB amlodipine besylate was correlated with decreased mortality[82]. In fact, CCBs were described to possess antiviral activity against several evolving viruses, including bunyaviruses, arenaviruses, and flaviviruses[82]. Furthermore, CCBs were described to possess anti-inflammatory ability to control patients' intracellular Ca^{2+} levels, including decreasing the death rate in septic animal models with severe inflammatory outcomes[83,84]. Severe inflammatory outcomes are described to be linked to a critical COVID-19 result [85]. Thus, it is plausible that CCBs may operate in a synergistic way by combining their antiviral efficacy with alleviation of inflammatory responses[85].

Complementing the present discussion, and in addition to Ca^{2+} signaling, the participation of cAMP signaling (Ca^{2+}/cAMP signaling) in the correlation between cancer, hypertension, diabetes, and dementia is considered.

CANCER, HYPERTENSION, DIABETES, AND DEMENTIA: PARTICIPATION OF CA^{2+}/CAMP SIGNALING

Fundamental mechanisms

Our reports on Ca^{2+}/cAMP signaling have recognized the participation of these cellular processes in regulating the release of both neurotransmitters and hormones, as well as the death of neurons and tumor growth[9,10,12,24,25,32-36,86,87]. Our studies proved that by decreasing the influx of Ca^{2+} *via* voltage activated Ca^{2+} channels (VACCs), adenylyl cyclases (ACs) are stimulated (thus increasing the levels of cAMP, and the Ca^{2+}/cAMP signaling interaction, Figure 1).

Considering this working model, CCBs-responses can be significantly increased through their pharmaceutical association with cAMP-enhancer agents [such as phosphodiesterase inhibitors]. The working model through which the release of both transmitter and hormone can be significantly augmented by regulating Ca^{2+}/cAMP signaling is related to (1) elevating the concentrations of transmitters and hormones in the secretory apparatus; and (2) enhancing the release of transmitters and hormones [10,12]. Actually, Ca^{2+} signaling is essential for supporting the release process: *Via* rising cAMP levels, this can augment the release of Ca^{2+} from endoplasmic reticulum (ER), thus increasing the release of transmitters and hormones. The timeline of the major events in Ca^{2+}/cAMP signaling can be found below (Table 1).

Additionally, a higher $[Ca^{2+}]c$ from critical dysregulations of Ca^{2+} signaling, such as an enhanced Ca^{2+} influx, has been linked to dementia, diabetes, hypertension, and cancer[9,10,12,24,25]. For instance, it was observed that L-type Ca^{2+} channels are significantly up-regulated in different types of cancer cells, contributing to abnormal cell proliferation[9,40-44]. The pharmaceutical modulation of these channels could then improve the therapeutics for antitumor purposes.

As well as cancer, dysregulations related to aging have also been detected in Ca^{2+} signaling pathways, stimulating the death of neurons, *e.g.*, an increase in intracellular Ca^{2+} levels, an augmented Ca^{2+} influx *via* the VACC and abnormalities in Ca^{2+} regulation in ryanodine and IP_3-sensitive Ca^{2+} stores[10,12,24].

Opposing Ca^{2+} signaling, stimulation of cAMP/protein kinase/cAMP-response element binding protein pathways can reduce both neuronal death and abnormal cell proliferation, thus resulting in anti-cancer and anti-dementia effects[88-91]. Thus, both the death of neurons and abnormal cell proliferation may also be a consequence of reduced activity of signaling pathways controlled by cAMP, as well as an increase in $[Ca^{2+}]c$, resulting from disruption of Ca^{2+}/cAMP signaling interactions. Indeed, there is a clear relationship between Ca^{2+} signaling, *e.g.*, increased Ca^{2+} signals, and inflammatory responses[92]. cAMP also modulates inflammatory responses: medicines which increase intracellular levels of cAMP can diminish the generation of pro-inflammatory factors, and enhance the generation of anti-inflammatory molecules[93]. Furthermore, whereas a physiological increase in the cytoplasmic concentration of Ca^{2+} is a significant trigger to release insulin, an abnormal elevation in $[Ca^{2+}]c$ could stimulate β-cell apoptosis, then decrease insulin levels, contributing to diabetes[9,12,25]. Together with Ca^{2+}, cAMP modulates the release of various hormones, as well as insulin from the pancreatic β-cells[51,52]. Although increasing cAMP levels, *e.g.*, *via* adrenaline, might stimulate the biosynthesis of hepatic glucose, increasing cAMP

Table 1 Timeline of the major events in Ca^{2+}/3'-5'-cyclic adenosine monophosphate signaling

	1970s	1980s and 1990s	2000s and 2010s	2019-2020
Major events	Verapamil paradoxically enhanced the contractions of smooth muscles, *e.g.*, rat vas deferens	Other CCBs (besides verapamil) also paradoxically enhanced the contractions of smooth muscles, *e.g.*, rat vas deferens	2013. Bergantin *et al*[32] discovered that the paradoxical increase in the contractions of smooth muscles, produced by CCBs, was due to an interaction of Ca^{2+}/cAMP signaling 2015-2016. Bergantin *et al*[24] proposed that the pharmacological manipulation of Ca^{2+}/cAMP signaling could be a new therapeutic strategy for increasing neurotransmission in psychiatric disorders, and producing neuroprotection in neurodegenerative diseases	Bergantin[12,25,86,87] discussed the involvement of Ca^{2+}/cAMP signaling in the pathogenesis of several diseases, including hypertension, diabetes, neurodegenerative diseases, asthma, and cancer
Articles indexed in PubMed (PMID)	PMID: 1143442	PMID: 3113986; PMID: 2466518	PMID: 23849429; PMID: 26516591; PMID: 27349146	PMID: 30117399; PMID: 30639385; PMID: 30771427; PMID: 30648516; PMID: 31291877; PMID: 31456527; PMID: 31995022; PMID: 32077833; PMID: 32186273; PMID: 32026774; PMID: 32065096; PMID: 32562933; PMID: 33210037; PMID: 33176668

CCBs: Ca^{2+} channel blockers; cAMP: 3'-5'-cyclic adenosine monophosphate.

Table 2 A list of several Ca^{2+} channel blockers and 3'-5'-cyclic adenosine monophosphate signaling-enhancer compounds

CCBs	cAMP signaling (enhancer compounds)
Verapamil	Rolipram
Nifedipine	3-isobutyl-1-methylxanthine (IBMX)
Diltiazem	Forskolin
Isradipine	Aminophylline
Amlodipine	Theophylline
Nicardipine	Paraxanthine

CCBs: Ca^{2+} channel blockers; cAMP: 3'-5'-cyclic adenosine monophosphate.

levels within pancreatic β-cells can stimulate insulin release. A start signal for the release of insulin is achieved through an increase in Ca^{2+} concentration, and after this signal is amplified *via* cAMP[53]. Besides the cellular effect of increasing the biosynthesis of insulin, cAMP is implicated in other cellular processes of β-cell, *e.g.*, stimulating both proliferation and differentiation of the cell, and by rescuing the cells from death[53]. These effects are summarized in Figure 2.

A medical correlation

Ca^{2+}/cAMP signaling has been highlighted as a protagonist in hypertension, cancer, diabetes, and dementia[9,10,12,24,25,32-36,86,87]. Considering the experience of our group in this field, our reports undoubtedly show that Ca^{2+} release from the ER can be induced by an increase in $[cAMP]_c$. Therefore, considering the participation of Ca^{2+}/cAMP signaling pathways in modulating the release of both neurotransmitters and hormones, as well as tumor growth and neurodegeneration, dysregulations of these signaling pathways can result in disorders such as hypertension, dementia, diabetes, and cancer[9,10,12,24,25,32-36,86,87].

In addition, several findings have confirmed that CCBs, despite their classical antihypertensive effect, can attenuate the symptoms of dementia, diabetes, and cancer [9,10,12,29,94-96]. Similar effects could be achieved by increasing [cAMP]c[9,10,12,24,88-90]. Please see Table 2.

Undeniably, an increase in the release of neurotransmitters, and a reduction in the death of neurons in the CNS (*e.g.*, limbic brain sites), could cause as a consequence a decrease in symptoms related to dementia, a working model that can be controlled by Ca^{2+} and cAMP signaling pathways[10,12,25,33]. Analogous to dementia[10,12,33],

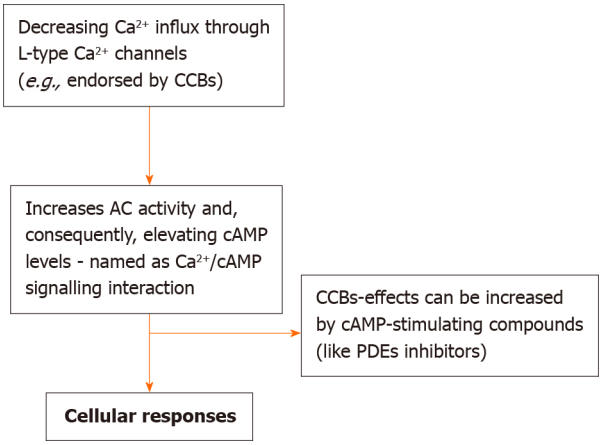

Figure 1 Pharmaceutical modulation of Ca^{2+}/3'-5'-cyclic adenosine monophosphate signaling. Decreasing Ca^{2+} influx through L-type Ca^{2+} channels, *e.g.*, endorsed by Ca^{2+} channel blockers (CCBs), enhances adenylyl cyclase activity (and consequently increases 3'-5'-cyclic adenosine monophosphate (cAMP) levels; identified as a Ca^{2+}/cAMP signaling interaction), and these effects of CCBs could be increased by cAMP-enhancer compounds (such as phosphodiesterase inhibitors). CCBs: Ca^{2+} channel blockers; cAMP: 3'-5'-cyclic adenosine monophosphate; AC: Adenylyl cyclase; PDEs: Phosphodiesterases.

Disruption of Ca^{2+}/cAMP signalling			
↑ **$[Ca^{2+}]_c$**		↓ **$[cAMP]_c$**	
Abnormal cellular consequences			
Increase of the neuronal death	Increase of the catecholamines´ release	Stimulation of abnormal cell proliferation	Increase of β-cell apoptosis and decrease of the insulin levels
Symptoms and clinical consequences			
Neurodegeneration and neuroinflammation	Sympathetic hyperactivity	Tumor growth	Dysregulations of the glucose homeostasis and stimulation of inflammatory processes
Disorders			
Dementia	Hypertension	Cancer	Diabetes

Figure 2 The Ca^{2+}/3'-5'-cyclic adenosine monophosphate signaling dysregulations and their consequences. Up arrow: Increasing; Down arrow: Decreasing. $[Ca^{2+}]_c$: Intracellular concentration of Ca^{2+}; $[cAMP]_c$: Intracellular concentration of 3'-5'-cyclic adenosine monophosphate.

discoveries have shown that CCBs can also mitigate the symptoms of both cancer and diabetes[9,12,94-96]. Reestablishing the dyshomeostasis associated with Ca^{2+} signaling is a working model for these CCBs-mentioned responses, reached due to intervening in the Ca^{2+}/cAMP signaling interactions. In fact, CCBs stimulate the activity of ACs, following an increase in $[cAMP]_c$, promoting Ca^{2+} release from the ER, ultimately inducing the release of both neurotransmitters and hormones, and decreasing the death of neurons and attenuating tumor growth. Considering that the link between diseases (cancer, diabetes, hypertension, and dementia) could be a consequence of persistent dysregulations of $[Ca^{2+}]_c$, the persistent increase in $[Ca^{2+}]_c$ might also disturb Ca^{2+}/cAMP signaling interactions.

CONCLUSION

Both Ca^{2+} and cAMP signaling pathways regulate the release of neurotransmitters and hormones, including those involved in neurodegeneration and tumor growth.

Furthermore, there is a clear relationship between Ca^{2+} signaling, *e.g.*, increased Ca^{2+} signals and inflammatory responses. cAMP also regulates pro- and anti-inflammatory responses. It is concluded that both signaling pathways play an important role in the link between diabetes and inflammatory diseases, thus impacting therapeutics including CCBs and medicines which increase the levels of cAMP. Finally, as COVID-19 is an emerging and rapidly evolving situation, it is also concluded that Ca^{2+} channel blockers could be useful for preventing Ca^{2+} signaling disruption due to COVID-19.

ACKNOWLEDGEMENTS

Dr. Leandro Bergantin would like to thank the reviewers and editors for their suggestions to improve this manuscript.

REFERENCES

1 **Lim S**, Yoo BK, Kim HS, Gilmore HL, Lee Y, Lee HP, Kim SJ, Letterio J, Lee HG. Amyloid-β precursor protein promotes cell proliferation and motility of advanced breast cancer. *BMC Cancer* 2014; **14**: 928 [PMID: 25491510 DOI: 10.1186/1471-2407-14-928]

2 **Wezyk M**, Szybinska A, Wojsiat J, Szczerba M, Day K, Ronnholm H, Kele M, Berdynski M, Peplonska B, Fichna JP, Ilkowski J, Styczynska M, Barczak A, Zboch M, Filipek-Gliszczynska A, Bojakowski K, Skrzypczak M, Ginalski K, Kabza M, Makalowska I, Barcikowska-Kotowicz M, Wojda U, Falk A, Zekanowski C. Overactive BRCA1 Affects Presenilin 1 in Induced Pluripotent Stem Cell-Derived Neurons in Alzheimer's Disease. *J Alzheimers Dis* 2018; **62**: 175-202 [PMID: 29439343 DOI: 10.3233/JAD-170830]

3 **Jin WS**, Bu XL, Liu YH, Shen LL, Zhuang ZQ, Jiao SS, Zhu C, Wang QH, Zhou HD, Zhang T, Wang YJ. Plasma Amyloid-Beta Levels in Patients with Different Types of Cancer. *Neurotox Res* 2017; **31**: 283-288 [PMID: 27913965 DOI: 10.1007/s12640-016-9682-9]

4 **Stocks T**, Van Hemelrijck M, Manjer J, Bjørge T, Ulmer H, Hallmans G, Lindkvist B, Selmer R, Nagel G, Tretli S, Concin H, Engeland A, Jonsson H, Stattin P. Blood pressure and risk of cancer incidence and mortality in the Metabolic Syndrome and Cancer Project. *Hypertension* 2012; **59**: 802-810 [PMID: 22353615 DOI: 10.1161/HYPERTENSIONAHA.111.189258]

5 **Stocks T**, Borena W, Strohmaier S, Bjørge T, Manjer J, Engeland A, Johansen D, Selmer R, Hallmans G, Rapp K, Concin H, Jonsson H, Ulmer H, Stattin P. Cohort Profile: The Metabolic syndrome and Cancer project (Me-Can). *Int J Epidemiol* 2010; **39**: 660-667 [PMID: 19380371 DOI: 10.1093/ije/dyp186]

6 **Andreotti G**, Boffetta P, Rosenberg PS, Berndt SI, Karami S, Menashe I, Yeager M, Chanock SJ, Zaridze D, Matteev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Rothman N, Brennan P, Chow WH, Moore LE. Variants in blood pressure genes and the risk of renal cell carcinoma. *Carcinogenesis* 2010; **31**: 614-620 [PMID: 20047954 DOI: 10.1093/carcin/bgp321]

7 **Corrao G**, Scotti L, Bagnardi V, Sega R. Hypertension, antihypertensive therapy and renal-cell cancer: a meta-analysis. *Curr Drug Saf* 2007; **2**: 125-133 [PMID: 18690958 DOI: 10.2174/157488607780598296]

8 **Seretis A**, Cividini S, Markozannes G, Tseretopoulou X, Lopez DS, Ntzani EE, Tsilidis KK. Association between blood pressure and risk of cancer development: a systematic review and meta-analysis of observational studies. *Sci Rep* 2019; **9**: 8565 [PMID: 31189941 DOI: 10.1038/s41598-019-45014-4]

9 **Bergantin LB**. Diabetes and cancer: Debating the link through Ca^{2+}/cAMP signalling. *Cancer Lett* 2019; **448**: 128-131 [PMID: 30771427 DOI: 10.1016/j.canlet.2019.02.017]

10 **Bergantin LB**, Caricati-Neto A. The "Calcium Paradox" and its Impact on Neurological and Psychiatric Diseases. Cambridge Scholars Publishing; 2018

11 **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]

12 **Bergantin LB**. Hypertension, Diabetes and Neurodegenerative Diseases: Is there a Clinical Link through the Ca2+/cAMP Signalling Interaction? *Curr Hypertens Rev* 2019; **15**: 32-39 [PMID: 30117399 DOI: 10.2174/1573402114666180817113242]

13 **Hotamisligil GS**, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; **259**: 87-91 [PMID: 7678183 DOI: 10.1126/science.7678183]

14 **Ogston D**, Mcandrew GM. Fibrinolysis in obesity. *Lancet* 1964; **2**: 1205-1207 [PMID: 14215560 DOI: 10.1016/s0140-6736(64)91042-6]

15 **Emerging Risk Factors Collaboration**, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 2010; **375**: 132-140

[PMID: 20031199 DOI: 10.1016/S0140-6736(09)61717-7]
16 **Ridker PM**, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997; **336**: 973-979 [PMID: 9077376 DOI: 10.1056/NEJM199704033361401]
17 **Duncan BB**, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G; Atherosclerosis Risk in Communities Study. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003; **52**: 1799-1805 [PMID: 12829649 DOI: 10.2337/diabetes.52.7.1799]
18 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545 [PMID: 11229684 DOI: 10.1016/S0140-6736(00)04046-0]
19 **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867 [PMID: 12490959 DOI: 10.1038/nature01322]
20 **Kinney JW**, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement (N Y)* 2018; **4**: 575-590 [PMID: 30406177 DOI: 10.1016/j.trci.2018.06.014]
21 **De Miguel C**, Rudemiller NP, Abais JM, Mattson DL. Inflammation and hypertension: new understandings and potential therapeutic targets. *Curr Hypertens Rep* 2015; **17**: 507 [PMID: 25432899 DOI: 10.1007/s11906-014-0507-z]
22 **Hemminki K**, Hemminki O, Försti A, Sundquist K, Sundquist J, Li X. Surveillance Bias in Cancer Risk After Unrelated Medical Conditions: Example Urolithiasis. *Sci Rep* 2017; **7**: 8073 [PMID: 28808281 DOI: 10.1038/s41598-017-08839-5]
23 **Sperling RA**, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR Jr, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; **7**: 280-292 [PMID: 21514248 DOI: 10.1016/j.jalz.2011.03.003]
24 **Bergantin LB**, Caricati-Neto A. Challenges for the pharmacological treatment of neurological and psychiatric disorders: Implications of the Ca(2+)/cAMP intracellular signalling interaction. *Eur J Pharmacol* 2016; **788**: 255-260 [PMID: 27349146 DOI: 10.1016/j.ejphar.2016.06.034]
25 **Bergantin LB**. Debating the "bidirectional link" between diabetes and depression through the Ca^{2+} /cAMP signalling: Off-label effects of Ca^{2+} channel blockers. *Pharmacol Res* 2019; **141**: 298-302 [PMID: 30639385 DOI: 10.1016/j.phrs.2019.01.008]
26 **Marques R**, Peres CG, Vaz CV, Gomes IM, Figueira MI, Cairrão E, Verde I, Maia CJ, Socorro S. 5α-Dihydrotestosterone regulates the expression of L-type calcium channels and calcium-binding protein regucalcin in human breast cancer cells with suppression of cell growth. *Med Oncol* 2015; **32**: 228 [PMID: 26255063 DOI: 10.1007/s12032-015-0676-x]
27 **Dalal PJ**, Muller WA, Sullivan DP. Endothelial Cell Calcium Signaling during Barrier Function and Inflammation. *Am J Pathol* 2020; **190**: 535-542 [PMID: 31866349 DOI: 10.1016/j.ajpath.2019.11.004]
28 **Vaz CV**, Rodrigues DB, Socorro S, Maia CJ. Effect of extracellular calcium on regucalcin expression and cell viability in neoplastic and non-neoplastic human prostate cells. *Biochim Biophys Acta* 2015; **1853**: 2621-2628 [PMID: 26171977 DOI: 10.1016/j.bbamcr.2015.07.006]
29 **Wu CL**, Wen SH. A 10-year follow-up study of the association between calcium channel blocker use and the risk of dementia in elderly hypertensive patients. *Medicine (Baltimore)* 2016; **95**: e4593 [PMID: 27512890 DOI: 10.1097/MD.0000000000004593]
30 **Tully PJ**, Peters R, Pérès K, Anstey KJ, Tzourio C. Effect of SSRI and calcium channel blockers on depression symptoms and cognitive function in elderly persons treated for hypertension: three city cohort study. *Int Psychogeriatr* 2018; **30**: 1345-1354 [PMID: 29559030 DOI: 10.1017/S1041610217002903]
31 **Yoshida J**, Ishibashi T, Nishio M. G1 cell cycle arrest by amlodipine, a dihydropyridine Ca2+ channel blocker, in human epidermoid carcinoma A431 cells. *Biochem Pharmacol* 2007; **73**: 943-953 [PMID: 17217918 DOI: 10.1016/j.bcp.2006.12.011]
32 **Bergantin LB**, Souza CF, Ferreira RM, Smaili SS, Jurkiewicz NH, Caricati-Neto A, Jurkiewicz A. Novel model for "calcium paradox" in sympathetic transmission of smooth muscles: role of cyclic AMP pathway. *Cell Calcium* 2013; **54**: 202-212 [PMID: 23849429 DOI: 10.1016/j.ceca.2013.06.004]
33 **Caricati-Neto A**, García AG, Bergantin LB. Pharmacological implications of the Ca(2+)/cAMP signaling interaction: from risk for antihypertensive therapy to potential beneficial for neurological and psychiatric disorders. *Pharmacol Res Perspect* 2015; **3**: e00181 [PMID: 26516591 DOI: 10.1002/prp2.181]
34 **Caricati-Neto A**, Bergantin LB. Pharmacological modulation of neural Ca2+/camp signaling interaction as therapeutic goal for treatment of Alzheimer's disease. *J Syst Integr Neurosci* 2017; **3** [DOI: 10.15761/JSIN.1000185]
35 **Caricati-Neto A**, Bergantin LB. The passion of a scientific discovery: the "calcium paradox" due to Ca2+/camp interaction. *J Syst Integr Neurosci* 2017; **3** [DOI: 10.15761/JSIN.1000186]
36 **Caricati-Neto A**, Bergantin LB. From a "eureka insight" to a novel potential therapeutic target to treat Parkinson's disease: The Ca2+/camp signalling interaction. *J Syst Integr Neurosci* 2017; **4** [DOI: 10.15761/JSIN.1000187]
37 **Bezprozvanny I**, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's

disease. *Trends Neurosci* 2008; **31**: 454-463 [PMID: 18675468 DOI: 10.1016/j.tins.2008.06.005]
38 **St George-Hyslop PH**, Petit A. Molecular biology and genetics of Alzheimer's disease. *C R Biol* 2005; **328**: 119-130 [PMID: 15770998 DOI: 10.1016/j.crvi.2004.10.013]
39 **Kale VP**, Amin SG, Pandey MK. Targeting ion channels for cancer therapy by repurposing the approved drugs. *Biochim Biophys Acta* 2015; **1848**: 2747-2755 [PMID: 25843679 DOI: 10.1016/j.bbamem.2015.03.034]
40 **Gomez-Ospina N**, Tsuruta F, Barreto-Chang O, Hu L, Dolmetsch R. The C terminus of the L-type voltage-gated calcium channel Ca(V)1.2 encodes a transcription factor. *Cell* 2006; **127**: 591-606 [PMID: 17081980 DOI: 10.1016/j.cell.2006.10.017]
41 **Dziegielewska B**, Gray LS, Dziegielewski J. T-type calcium channels blockers as new tools in cancer therapies. *Pflugers Arch* 2014; **466**: 801-810 [PMID: 24449277 DOI: 10.1007/s00424-014-1444-z]
42 **Ohkubo T**, Yamazaki J. T-type voltage-activated calcium channel Cav3.1, but not Cav3.2, is involved in the inhibition of proliferation and apoptosis in MCF-7 human breast cancer cells. *Int J Oncol* 2012; **41**: 267-275 [PMID: 22469755 DOI: 10.3892/ijo.2012.1422]
43 **Gackière F**, Bidaux G, Delcourt P, Van Coppenolle F, Katsogiannou M, Dewailly E, Bavencoffe A, Van Chuoï-Mariot MT, Mauroy B, Prevarskaya N, Mariot P. CaV3.2 T-type calcium channels are involved in calcium-dependent secretion of neuroendocrine prostate cancer cells. *J Biol Chem* 2008; **283**: 10162-10173 [PMID: 18230611 DOI: 10.1074/jbc.M707159200]
44 **Latour I**, Louw DF, Beedle AM, Hamid J, Sutherland GR, Zamponi GW. Expression of T-type calcium channel splice variants in human glioma. *Glia* 2004; **48**: 112-119 [PMID: 15378657 DOI: 10.1002/glia.20063]
45 **Yamaguchi M**, Osuka S, Shoji M, Weitzmann MN, Murata T. Survival of lung cancer patients is prolonged with higher regucalcin gene expression: suppressed proliferation of lung adenocarcinoma A549 cells in vitro. *Mol Cell Biochem* 2017; **430**: 37-46 [PMID: 28181135 DOI: 10.1007/s11010-017-2952-x]
46 **Yamaguchi M**. Suppressive role of regucalcin in liver cell proliferation: involvement in carcinogenesis. *Cell Prolif* 2013; **46**: 243-253 [PMID: 23692083 DOI: 10.1111/cpr.12036]
47 **Huang MY**, Wang HM, Chang HJ, Hsiao CP, Wang JY, Lin SR. Overexpression of S100B, TM4SF4, and OLFM4 genes is correlated with liver metastasis in Taiwanese colorectal cancer patients. *DNA Cell Biol* 2012; **31**: 43-49 [PMID: 22011044 DOI: 10.1089/dna.2011.1264]
48 **Miranda-Ferreira R**, de Pascual R, de Diego AM, Caricati-Neto A, Gandía L, Jurkiewicz A, García AG. Single-vesicle catecholamine release has greater quantal content and faster kinetics in chromaffin cells from hypertensive, as compared with normotensive, rats. *J Pharmacol Exp Ther* 2008; **324**: 685-693 [PMID: 17962518 DOI: 10.1124/jpet.107.128819]
49 **Miranda-Ferreira R**, de Pascual R, Caricati-Neto A, Gandía L, Jurkiewicz A, García AG. Role of the endoplasmic reticulum and mitochondria on quantal catecholamine release from chromaffin cells of control and hypertensive rats. *J Pharmacol Exp Ther* 2009; **329**: 231-240 [PMID: 19131584 DOI: 10.1124/jpet.108.147413]
50 **McCrimmon RJ**, Ryan CM, Frier BM. Diabetes and cognitive dysfunction. *Lancet* 2012; **379**: 2291-2299 [PMID: 22683129 DOI: 10.1016/S0140-6736(12)60360-2]
51 **Henquin JC**. The interplay between cyclic AMP and ions in the stimulus-secretion coupling in pancreatic B-cells. *Arch Int Physiol Biochim* 1985; **93**: 37-48 [PMID: 2409943 DOI: 10.3109/13813458509104514]
52 **Bratanova-Tochkova TK**, Cheng H, Daniel S, Gunawardana S, Liu YJ, Mulvaney-Musa J, Schermerhorn T, Straub SG, Yajima H, Sharp GW. Triggering and augmentation mechanisms, granule pools, and biphasic insulin secretion. *Diabetes* 2002; **51** Suppl 1: S83-S90 [PMID: 11815463 DOI: 10.2337/diabetes.51.2007.s83]
53 **Tengholm A**. Cyclic AMP dynamics in the pancreatic β-cell. *Ups J Med Sci* 2012; **117**: 355-369 [PMID: 22970724 DOI: 10.3109/03009734.2012.724732]
54 **van der Willik KD**, Schagen SB, Ikram MA. Cancer and dementia: Two sides of the same coin? *Eur J Clin Invest* 2018; **48**: e13019 [PMID: 30112764 DOI: 10.1111/eci.13019]
55 **Ahles TA**, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. *Nat Rev Cancer* 2007; **7**: 192-201 [PMID: 17318212 DOI: 10.1038/nrc2073]
56 **Weycker D**, Nichols GA, O'Keeffe-Rosetti M, Edelsberg J, Vincze G, Khan ZM, Oster G. Excess risk of diabetes in persons with hypertension. *J Diabetes Complications* 2009; **23**: 330-336 [PMID: 18513997 DOI: 10.1016/j.jdiacomp.2008.04.002]
57 **Cheung BM**, Li C. Diabetes and hypertension: is there a common metabolic pathway? *Curr Atheroscler Rep* 2012; **14**: 160-166 [PMID: 22281657 DOI: 10.1007/s11883-012-0227-2]
58 **Cheung BM**. The hypertension-diabetes continuum. *J Cardiovasc Pharmacol* 2010; **55**: 333-339 [PMID: 20422737 DOI: 10.1097/fjc.0b013e3181d26430]
59 **Landsberg L**, Molitch M. Diabetes and hypertension: pathogenesis, prevention and treatment. *Clin Exp Hypertens* 2004; **26**: 621-628 [PMID: 15702616 DOI: 10.1081/ceh-200031945]
60 **Gress TW**, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. *N Engl J Med* 2000; **342**: 905-912 [PMID: 10738048 DOI: 10.1056/NEJM200003303421301]
61 **Munshi M**, Grande L, Hayes M, Ayres D, Suhl E, Capelson R, Lin S, Milberg W, Weinger K. Cognitive dysfunction is associated with poor diabetes control in older adults. *Diabetes Care* 2006; **29**: 1794-1799 [PMID: 16873782 DOI: 10.2337/dc06-0506]
62 **Sinclair AJ**, Girling AJ, Bayer AJ. Cognitive dysfunction in older subjects with diabetes mellitus:

impact on diabetes self-management and use of care services. All Wales Research into Elderly (AWARE) Study. *Diabetes Res Clin Pract* 2000; **50**: 203-212 [PMID: 11106835 DOI: 10.1016/s0168-8227(00)00195-9]

63 **Alencar RC**, Cobas RA, Gomes MB. Assessment of cognitive status in patients with type 2 diabetes through the Mini-Mental Status Examination: a cross-sectional study. *Diabetol Metab Syndr* 2010; **2**: 10 [PMID: 20205826 DOI: 10.1186/1758-5996-2-10]

64 **Feinkohl I**, Keller M, Robertson CM, Morling JR, Williamson RM, Nee LD, McLachlan S, Sattar N, Welsh P, Reynolds RM, Russ TC, Deary IJ, Strachan MW, Price JF; Edinburgh Type 2 Diabetes Study (ET2DS) Investigators. Clinical and subclinical macrovascular disease as predictors of cognitive decline in older patients with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 2013; **36**: 2779-2786 [PMID: 23579182 DOI: 10.2337/dc12-2241]

65 **Zhao Q**, Roberts RO, Ding D, Cha R, Guo Q, Meng H, Luo J, Machulda MM, Shane Pankratz V, Wang B, Christianson TJ, Aakre JA, Knopman DS, Boeve BF, Hong Z, Petersen RC; Shanghai Aging Study (SAS); Mayo Clinic Study of Aging (MCSA). Diabetes is Associated with Worse Executive Function in Both Eastern and Western Populations: Shanghai Aging Study and Mayo Clinic Study of Aging. *J Alzheimers Dis* 2015; **47**: 167-176 [PMID: 26402765 DOI: 10.3233/JAD-150073]

66 **Qiu C**, Sigurdsson S, Zhang Q, Jonsdottir MK, Kjartansson O, Eiriksdottir G, Garcia ME, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Diabetes, markers of brain pathology and cognitive function: the Age, Gene/Environment Susceptibility-Reykjavik Study. *Ann Neurol* 2014; **75**: 138-146 [PMID: 24243491 DOI: 10.1002/ana.24063]

67 **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Münch G, Wood AG, Forbes J, Greenaway TM, Pearson S, Srikanth V. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care* 2013; **36**: 4036-4042 [PMID: 23939539 DOI: 10.2337/dc13-0143]

68 **Kooistra M**, Geerlings MI, van der Graaf Y, Mali WP, Vincken KL, Kappelle LJ, Muller M, Biessels GJ; SMART-MR Study Group. Vascular brain lesions, brain atrophy, and cognitive decline. The Second Manifestations of ARTerial disease--Magnetic Resonance (SMART-MR) study. *Neurobiol Aging* 2014; **35**: 35-41 [PMID: 23932882 DOI: 10.1016/j.neurobiolaging.2013.07.004]

69 **van Elderen SG**, de Roos A, de Craen AJ, Westendorp RG, Blauw GJ, Jukema JW, Bollen EL, Middelkoop HA, van Buchem MA, van der Grond J. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology* 2010; **75**: 997-1002 [PMID: 20837967 DOI: 10.1212/WNL.0b013e3181f25f06]

70 **Whitmer RA**. Type 2 diabetes and risk of cognitive impairment and dementia. *Curr Neurol Neurosci Rep* 2007; **7**: 373-380 [PMID: 17764626 DOI: 10.1007/s11910-007-0058-7]

71 **Guariguata L**, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014; **103**: 137-149 [PMID: 24630390 DOI: 10.1016/j.diabres.2013.11.002]

72 **Bruce DG**, Casey GP, Grange V, Clarnette RC, Almeida OP, Foster JK, Ives FJ, Davis TM; Fremantle Cognition in Diabetes Study. Cognitive impairment, physical disability and depressive symptoms in older diabetic patients: the Fremantle Cognition in Diabetes Study. *Diabetes Res Clin Pract* 2003; **61**: 59-67 [PMID: 12849924 DOI: 10.1016/s0168-8227(03)00084-6]

73 **Watson GS**, Craft S. The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs* 2003; **17**: 27-45 [PMID: 12467491 DOI: 10.2165/00023210-200317010-00003]

74 **Yaffe K**, Blackwell T, Whitmer RA, Krueger K, Barrett Connor E. Glycosylated hemoglobin level and development of mild cognitive impairment or dementia in older women. *J Nutr Health Aging* 2006; **10**: 293-295 [PMID: 16886099]

75 **Kodl CT**, Seaquist ER. Cognitive dysfunction and diabetes mellitus. *Endocr Rev* 2008; **29**: 494-511 [PMID: 18436709 DOI: 10.1210/er.2007-0034]

76 **Cunnane S**, Nugent S, Roy M, Courchesne-Loyer A, Croteau E, Tremblay S, Castellano A, Pifferi F, Bocti C, Paquet N, Begdouri H, Bentourkia M, Turcotte E, Allard M, Barberger-Gateau P, Fulop T, Rapoport SI. Brain fuel metabolism, aging, and Alzheimer's disease. *Nutrition* 2011; **27**: 3-20 [PMID: 21035308 DOI: 10.1016/j.nut.2010.07.021]

77 **Cunnane SC**, Plourde M, Pifferi F, Bégin M, Féart C, Barberger-Gateau P. Fish, docosahexaenoic acid and Alzheimer's disease. *Prog Lipid Res* 2009; **48**: 239-256 [PMID: 19362576 DOI: 10.1016/j.plipres.2009.04.001]

78 **Yassine HN**, Croteau E, Rawat V, Hibbeln JR, Rapoport SI, Cunnane SC, Umhau JC. DHA brain uptake and APOE4 status: a PET study with [1-^{11}C]-DHA. *Alzheimers Res Ther* 2017; **9**: 23 [PMID: 28335828 DOI: 10.1186/s13195-017-0250-1]

79 **Croteau E**, Castellano CA, Richard MA, Fortier M, Nugent S, Lepage M, Duchesne S, Whittingstall K, Turcotte ÉE, Bocti C, Fülöp T, Cunnane SC. Ketogenic Medium Chain Triglycerides Increase Brain Energy Metabolism in Alzheimer's Disease. *J Alzheimers Dis* 2018; **64**: 551-561 [PMID: 29914035 DOI: 10.3233/JAD-180202]

80 **Abdi A**, Jalilian M, Sarbarzeh PA, Vlaisavljevic Z. Diabetes and COVID-19: A systematic review on the current evidences. *Diabetes Res Clin Pract* 2020; **166**: 108347 [PMID: 32711003 DOI: 10.1016/j.diabres.2020.108347]

81 **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]
82 **Zhang LK**, Sun Y, Zeng H, Wang Q, Jiang X, Shang WJ, Wu Y, Li S, Zhang YL, Hao ZN, Chen H, Jin R, Liu W, Li H, Peng K, Xiao G. Calcium channel blocker amlodipine besylate therapy is associated with reduced case fatality rate of COVID-19 patients with hypertension. *Cell Discov* 2020; **6**: 96 [PMID: 33349633 DOI: 10.1038/s41421-020-00235-0]
83 **Silva IVG**, de Figueiredo RC, Rios DRA. Effect of Different Classes of Antihypertensive Drugs on Endothelial Function and Inflammation. *Int J Mol Sci* 2019; **20** [PMID: 31337127 DOI: 10.3390/ijms20143458]
84 **D'Elia JA**, Weinrauch LA. Calcium Ion Channels: Roles in Infection and Sepsis Mechanisms of Calcium Channel Blocker Benefits in Immunocompromised Patients at Risk for Infection. *Int J Mol Sci* 2018; **19** [PMID: 30134544 DOI: 10.3390/ijms19092465]
85 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]
86 **Bergantin LB**. The Interplay Between Asthma and Other Diseases: Role of Ca2+/cAMP Signalling. *Endocr Metab Immune Disord Drug Targets* 2020; **20**: 321-327 [PMID: 31456527 DOI: 10.2174/1871530319666190828145854]
87 **Bergantin LB**. Diabetes and Parkinson's Disease: Debating the Link Through Ca2+/cAMP Signalling. *Curr Diabetes Rev* 2020; **16**: 238-241 [PMID: 31291877 DOI: 10.2174/1573399815666190711113644]
88 **Fajardo AM**, Piazza GA, Tinsley HN. The role of cyclic nucleotide signaling pathways in cancer: targets for prevention and treatment. *Cancers (Basel)* 2014; **6**: 436-458 [PMID: 24577242 DOI: 10.3390/cancers6010436]
89 **Drees M**, Zimmermann R, Eisenbrand G. 3',5'-Cyclic nucleotide phosphodiesterase in tumor cells as potential target for tumor growth inhibition. *Cancer Res* 1993; **53**: 3058-3061 [PMID: 8391385]
90 **Xiao L**, O'Callaghan JP, O'Donnell JM. Effects of repeated treatment with phosphodiesterase-4 inhibitors on cAMP signaling, hippocampal cell proliferation, and behavior in the forced-swim test. *J Pharmacol Exp Ther* 2011; **338**: 641-647 [PMID: 21566211 DOI: 10.1124/jpet.111.179358]
91 **Uddin MS**, Stachowiak A, Mamun AA, Tzvetkov NT, Takeda S, Atanasov AG, Bergantin LB, Abdel-Daim MM, Stankiewicz AM. Autophagy and Alzheimer's Disease: From Molecular Mechanisms to Therapeutic Implications. *Front Aging Neurosci* 2018; **10**: 04 [PMID: 29441009 DOI: 10.3389/fnagi.2018.00004]
92 **Ribeiro CM**. The role of intracellular calcium signals in inflammatory responses of polarised cystic fibrosis human airway epithelia. *Drugs R D* 2006; **7**: 17-31 [PMID: 16620134 DOI: 10.2165/00126839-200607010-00002]
93 **Raker VK**, Becker C, Steinbrink K. The cAMP Pathway as Therapeutic Target in Autoimmune and Inflammatory Diseases. *Front Immunol* 2016; **7**: 123 [PMID: 27065076 DOI: 10.3389/fimmu.2016.00123]
94 **Novotny M**, Klimova B, Valis M. Nitrendipine and Dementia: Forgotten Positive Facts? *Front Aging Neurosci* 2018; **10**: 418 [PMID: 30618724 DOI: 10.3389/fnagi.2018.00418]
95 **Takada K**, Kashiwagi S, Asano Y, Goto W, Takahashi K, Fujita H, Takashima T, Tomita S, Hirakawa K, Ohira M. Verification of the effects of calcium channel blockers on the immune microenvironment of breast cancer. *BMC Cancer* 2019; **19**: 615 [PMID: 31234828 DOI: 10.1186/s12885-019-5828-5]
96 **Taghizadehghalehjoughi A**, Sezen S, Hacimuftuoglu A, Güllüce M. Vincristine combination with Ca^{+2} channel blocker increase antitumor effects. *Mol Biol Rep* 2019; **46**: 2523-2528 [PMID: 30903573 DOI: 10.1007/s11033-019-04706-w]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 780-785

DOI: 10.4239/wjd.v12.i6.780

ISSN 1948-9358 (online)

MINIREVIEWS

Effect of COVID-19 on management of type 1 diabetes: Pushing the boundaries of telemedical healthcare

Ines Bilic Curcic, Maja Cigrovski Berkovic, Tomislav Kizivat, Silvija Canecki Varzic, Robert Smolic, Martina Smolic

ORCID number: Ines Bilic Curcic 0000-0002-8861-5987; Maja Cigrovski Berkovic 0000-0003-0750-9785; Tomislav Kizivat 0000-0002-2523-6007; Silvija Canecki Varzic 0000-0001-9535-7915; Robert Smolic 0000-0003-4614-4344; Martina Smolic 0000-0002-6867-826X.

Author contributions: Bilic Curcic I, Smolic M, and Smolic R were responsible for the concept and design; Bilic Curcic I, Cigrovski Berkovic M were responsible for drafting the manuscript; Kizivat T was responsible for visualizations; Smolic M, Smolic R, Kizivat T, and Canecki Varzic S were responsible for critical revision of the manuscript.

Supported by Ines Bilić-Ćurčić, No. ZUP2018-90.

Conflict-of-interest statement: There is no conflict of interest associated with the senior author or other coauthors.

Ines Bilic Curcic, Silvija Canecki Varzic, Department of Endocrinology, University Hospital Osijek, Osijek 31000, Croatia

Ines Bilic Curcic, Martina Smolic, Department of Pharmacology, Faculty of Medicine Osijek, Osijek 31000, Croatia

Maja Cigrovski Berkovic, Department of Endocrinology, Diabetes, Metabolism and Clinical Pharmacology, University Hospital Dubrava, Zagreb 10000, Croatia

Maja Cigrovski Berkovic, Department of Kinesiological Anthropology and Methodology, Faculty of Kinesiology, University of Zagreb, Zagreb 10000, Croatia

Tomislav Kizivat, Clinical Institute of Nuclear Medicine and Radiation Protection, University Hospital Osijek, Osijek 31000, Croatia

Tomislav Kizivat, Department of Nuclear Medicine and Oncology, Faculty of Medicine Osijek, Osijek 31000, Croatia

Silvija Canecki Varzic, Department of Internal Medicine, Family Medicine, and History, Faculty of Medicine Osijek, Osijek 31000, Croatia

Robert Smolic, Department of Pathophysiology, Faculty of Medicine Osijek, Osijek 31000, Croatia

Robert Smolic, Department of Pathophysiology, Physiology and Immunology, Faculty of Dental Medicine and Health, Osijek 31000, Croatia

Martina Smolic, Department of Pharmacology and Biochemistry, Faculty of Dental Medicine and Health, Osijek 31000, Croatia

Corresponding author: Martina Smolic, MD, PhD, Associate Professor, Department for Pharmacology, Faculty of Medicine Osijek, J Huttlera 4, Osijek 31000, Croatia.
martina.smolic@mefos.hr

Abstract

The new coronavirus disease 2019 (COVID-19) pandemic posed a great burden on health care systems worldwide and is an enormous and real obstacle in providing needed health care to patients with chronic diseases such as diabetes. Parallel to COVID-19, there have been great advances in technology used for management of

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Croatia

Peer-review report's scientific quality classification

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

Received: January 13, 2021
Peer-review started: January 13, 2021
First decision: April 20, 2021
Revised: April 23, 2021
Accepted: May 19, 2021
Article in press: May 19, 2021
Published online: June 15, 2021

P-Reviewer: Bansal A, Liu YC
S-Editor: Zhang L
L-Editor: Filipodia
P-Editor: Ma YJ

type 1 diabetes, primarily insulin pumps, sensors, integrated and closed loop systems, ambulatory glucose profile software, and smart phone apps providing necessary essentials for telemedicine implementation right at the beginning of the COVID-19 pandemic. The results of these remote interventions are reassuring in terms of glycemic management and hemoglobin A1c reductions. However, data on long-term outcomes and cost reductions are missing as well as proper technical infrastructure and government health policy support.

Key Words: Diabetes management; Telemedicine; COVID-19; Diabetes type 1

Core Tip: Mortality and morbidity rates increased during the coronavirus disease 2019 pandemic partially due to disruption in health care delivery. The implementation of telemedicine imposes itself as a logical solution given technical devices and apps already available in the management of type 1 diabetes. Presently available data are scarce but encouraging regarding glycemic control in long standing type 1 diabetes and new onset type 1 diabetes and minimizing acute complications.

Citation: Bilic Curcic I, Cigrovski Berkovic M, Kizivat T, Canecki Varzic S, Smolic R, Smolic M. Effect of COVID-19 on management of type 1 diabetes: Pushing the boundaries of telemedical healthcare. *World J Diabetes* 2021; 12(6): 780-785
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/780.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.780

INTRODUCTION

Diabetes and coronavirus disease 2019-aftermath to be seen

The coronavirus disease 2019 (COVID-19) pandemic is one of the biggest challenges humanity has ever encountered with unfathomable aftermaths on all aspects of our lives including the health care system or rather the disruption of health care delivery.

Interestingly, diabetes and COVID-19 are both pandemics with distinct opposite features. The COVID-19 pandemic is a newly emerged infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In the short period of time, it caused over 1850941 deaths[1] rising as a global emergency and changing the face of health care provision in a short period of time. On the other hand, diabetes is a slow pandemic, and one of the leading causes of mortality and morbidity worldwide responsible for over 42 million deaths in 2019[2].

Moreover, clinical presentation of SARS-CoV-2 infection tends to be more severe with increased mortality rates in people with type 1 and type 2 diabetes, especially those with poor glycemic regulation and accompanying comorbidities such as obesity, kidney impairment, and cardiovascular disease[3,4]. An increase in mortality rates in diabetic patients, both type 1 and type 2, has been observed in the first 3 mo of 2020 compared with the same period in the 5 years prior (from 2014 to 2019), which could be a consequence of inadequate health care as well as COVID-19[3] emphasizing an urgent need for practical solutions in remote outpatient health care.

An emerging role of remote outpatient care in diabetes management

If anything, the COVID-19 pandemic accelerated the implementation of telemedicine worldwide due to mandatory social distancing, and many patients' health care providers were discovering benefits attached to remote health care[5]. Patients can receive guidance and consulting from their homes thus avoiding a potential virus threat, saving time and costs of travel and parking, which is especially convenient for children and the working population.

Diabetes type 1 and telemedicine-a big step forward

This form of diabetes management is particularly appropriate for type 1 patients already using available software, such as Dexcom, Care Link, or LabVIEW, able to

generate ambulatory glucose profile reports, and using smart insulin pens thus allowing remote monitoring of glucose management and providing consultations based on available data *via* phone, video calls, or smart phone applications[6-8].

Indeed, the digital revolution commenced in the type 1 community starting with insulin pumps, advancing with sensors, integrated and closed loop systems, ambulatory glucose profile software, and smart phone apps procuring necessary essentials for swift and timely telemedicine implementation right at the beginning of the COVID-19 pandemic[9].

This was clearly shown in a study performed in Italy during the COVID-19 lockdown including people with type 1 diabetes using the hybrid closed loop demonstrating improved glycemic control probably due to the availability of telemedicine and more active engagement of patients in glycemic management[10].

A study conducted on type 1 diabetes patients from 89 countries encompassing 7477 survey responses showed that 30% believed their healthcare access was negatively affected, while 28% received remote care through telephone (72%) or video calls (28%). The majority of those patients considered teleconsulting useful, and hemoglobin A1c levels positively correlated with affirmative attitude towards telemedicine[11].

Type 2 diabetes and telemedicine-limited experience in the COVID-19 era

In the pre-COVID-19 era, virtual consultations have proven useful, effective, and accessible in type 2 diabetes management compared to face-to-face visits[12,13]. Still, outcomes in terms of glycated hemoglobin vary by studies. For instance, Cochrane meta-analysis of 21 studies comparing standard care to telemedicine in diabetic patients demonstrated inconsistent results in hemoglobin A1c improvement but a better effect on low density lipoprotein and blood pressure levels[14]. Another study showed improvement in hemoglobin A1c levels. However, strong technical support was engaged including connected devices such as continuous glucose monitoring, remote lifestyle coaching, and clinical support with a mobile app, which are not usually on disposal for type 2 diabetes patients[15].

Data on telemedicine and type 2 diabetes in the COVID-19 era are still lacking. In a recently published study including 763 type 1 and 619 type 2 diabetics, about 40% of patients stated that all of their diabetes visits were cancelled or postponed, 40% were switched to telehealth consultations, while half reported lower overall satisfaction with these visits[16].

Managing new onset diabetes and acute complications in COVID-19 via telemedicine

Infection with SARS-CoV-2 causes an inexplicable rise in glycemia, probably due to direct toxic effects of the virus itself and wide expression of angiotensin converting enzyme 2 on islet cells[17,18] presenting with acute hyperglycemia followed by ketosis or even ketoacidosis requiring an emergency room visit even in previously well-controlled patients[19,20].

Telemedicine is allowing a continuous and remote communication between patients and their health care provider and in terms of COVID-19-induced acute hyperglycemia offers the only solution in outpatient glycemic management. In this way, consulting a patient on timely ketone screening and suitable actions could prevent development of ketosis and diabetic ketoacidosis and relive a burden on hospitals or at least ensure apt emergency room visits[21].

Recently, two case reports were published, one adult and the other pediatric, where telemedicine was effectively applied in all aspects of type 1 diabetes management, consultation, education, and monitoring through available software to generate ambulatory glucose profiles and using a combination of e-mail, Internet *via* Zoom, and telephone calls[22].

Future perspectives in telemedicine implementation

The major obstacle in telemedicine implementation are technical support issues and government reimbursement policies, which differ by country. Structured background for integration and reimbursement in most countries is missing. There are two options presented, one involving private providers depending on private insurance and the other based on free applications such as WhatsApp, Skype, or Zoom that are not in accordance with health data privacy conditions and are not an integrated part of health care registries[23]. In most countries, health insurance covers the costs of technical devices in the management of type 1 diabetes, which is not the case for type 2 diabetes. Precisely for this reason telemedicine is the most widely used in long standing type 1 diabetes management but also has potential in new onset type 1 diabetes and prevention of acute complications, especially important in the COVID-19

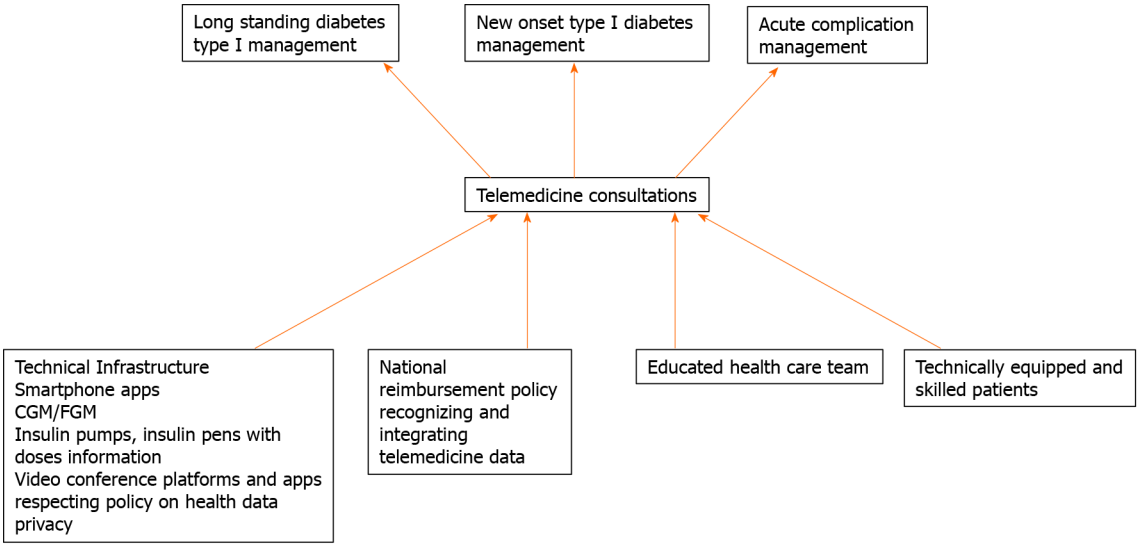

Figure 1 Essential requirements for successful implementation of telemedicine in the management of type 1 diabetes. CGM: Continuous glucose monitoring; FGM: Flash glucose monitoring.

era (Figure 1).

Downloading data from devices is a weak link in wider implementation of telemedicine because the older population is not skilled enough or do not have technical support necessary to prepare reports for consults. Unfortunately, this population in particular could benefit the most from remote consulting due to vulnerability to SARS-CoV-2 and other infections, walking disabilities, and poorer socioeconomic status. In addition, the majority of those patients do not have smart phones and do not use the internet frequently. Thus, improvements in user support services are necessary at this stage to resolve issues in service delivery[5].

The main question is could telemedicine replace face-to-face visits? One could argue that even if we have necessary data regarding glycemic management, we still could not perform a physical exam in order to evaluate cardiovascular health or polyneuropathy. It should be emphasized that telemedicine in retinopathy screening has been long recognized[24]. On the other hand, telemedicine and constant contact with patients enables physicians to act in time, to give advice regarding hypo- or hyperglycemia, adjust insulin doses, and provide proper actions in case of emergencies.

The potential in cost reductions and advancements of health care are plausible and supported by a recently published meta-analysis including 8 studies investigating a role of telemedicine in the COVID-19 pandemic confirming that telehealth care improves accessibility of health services[25]. However, there are no definite reports on long-term outcomes or cost reduction necessary for creating government health care policies as well as building technical infrastructure.

CONCLUSION

Nonetheless, virtual consultations and/or clinics are inevitable and essential in providing healthcare in this pandemic, securing communication between type 2 diabetes patients and health care providers necessary in supporting self-management. Based on present data, technical infrastructure is imperative in delivering high quality consultations ensuring patient satisfaction.

REFERENCES

1 **Worldometer**. COVID-19 coronavirus pandemic 2021. [cited 1 January 2021]. Available from: www.worldometers.info/coronavirus/

2 **International Diabetes Federation**. IDF Diabetes Atlas, 8th ed. Brussels, Belgium: 2019. [cited May 4, 2020]. Available from: www.idf.org/e-library/epidemiology-research/diabetes-atlas/134-idf-diabetes-atlas-8th-edition.html

3 **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]

4 **Williamson EJ**, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, Curtis HJ, Mehrkar A, Evans D, Inglesby P, Cockburn J, McDonald HI, MacKenna B, Tomlinson L, Douglas IJ, Rentsch CT, Mathur R, Wong AYS, Grieve R, Harrison D, Forbes H, Schultze A, Croker R, Parry J, Hester F, Harper S, Perera R, Evans SJW, Smeeth L, Goldacre B. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020; **584**: 430-436 [PMID: 32640463 DOI: 10.1038/s41586-020-2521-4]

5 **Wake DJ**, Gibb FW, Kar P, Kennon B, Klonoff DC, Rayman G, Rutter MK, Sainsbury C, Semple RK. ENDOCRINOLOGY IN THE TIME OF COVID-19: Remodelling diabetes services and emerging innovation. *Eur J Endocrinol* 2020; **183**: G67-G77 [PMID: 32508313 DOI: 10.1530/EJE-20-0377]

6 **Battelino T**, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, Bosi E, Buckingham BA, Cefalu WT, Close KL, Cobelli C, Dassau E, DeVries JH, Donaghue KC, Dovc K, Doyle FJ 3rd, Garg S, Grunberger G, Heller S, Heinemann L, Hirsch IB, Hovorka R, Jia W, Kordonouri O, Kovatchev B, Kowalski A, Laffel L, Levine B, Mayorov A, Mathieu C, Murphy HR, Nimri R, Nørgaard K, Parkin CG, Renard E, Rodbard D, Saboo B, Schatz D, Stoner K, Urakami T, Weinzimer SA, Phillip M. Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range. *Diabetes Care* 2019; **42**: 1593-1603 [PMID: 31177185 DOI: 10.2337/dci19-0028]

7 **Sangave NA**, Aungst TD, Patel DK. Smart Connected Insulin Pens, Caps, and Attachments: A Review of the Future of Diabetes Technology. *Diabetes Spectr* 2019; **32**: 378-384 [PMID: 31798296 DOI: 10.2337/ds18-0069]

8 **Iyengar K**, Upadhyaya GK, Vaishya R, Jain V. COVID-19 and applications of smartphone technology in the current pandemic. *Diabetes Metab Syndr* 2020; **14**: 733-737 [PMID: 32497963 DOI: 10.1016/j.dsx.2020.05.033]

9 **Danne T**, Limbert C. COVID-19, type 1 diabetes, and technology: why paediatric patients are leading the way. *Lancet Diabetes Endo* 2020; **8**: 465-467 [DOI: 10.1016/S2213-8587(20)30155-8]

10 **Longo M**, Caruso P, Petrizzo M, Castaldo F, Sarnataro A, Gicchino M, Bellastella G, Esposito K, Maiorino MI. Glycemic control in people with type 1 diabetes using a hybrid closed loop system and followed by telemedicine during the COVID-19 pandemic in Italy. *Diabetes Res Clin Pract* 2020; **169**: 108440 [PMID: 32926958 DOI: 10.1016/j.diabres.2020.108440]

11 **Scott SN**, Fontana FY, Züger T, Laimer M, Stettler C. Use and perception of telemedicine in people with type 1 diabetes during the COVID-19 pandemic-Results of a global survey. *Endocrinol Diabetes Metab* 2021; **4**: e00180 [PMID: 33532617 DOI: 10.1002/edm2.180]

12 **Armstrong N**, Hearnshaw H, Powell J, Dale J. Stakeholder perspectives on the development of a virtual clinic for diabetes care: qualitative study. *J Med Internet Res* 2007; **9**: e23 [PMID: 17942385 DOI: 10.2196/jmir.9.3.e23]

13 **Greenhalgh T**, Vijayaraghavan S, Wherton J, Shaw S, Byrne E, Campbell-Richards D, Bhattacharya S, Hanson P, Ramoutar S, Gutteridge C, Hodkinson I, Collard A, Morris J. Virtual online consultations: advantages and limitations (VOCAL) study. *BMJ Open* 2016; **6**: e009388 [PMID: 26826147 DOI: 10.1136/bmjopen-2015-009388]

14 **Flodgren G**, Rachas A, Farmer AJ, Inzitari M, Shepperd S. Interactive telemedicine: effects on professional practice and health care outcomes. *Cochrane Database Syst Rev* 2015; CD002098 [PMID: 26343551 DOI: 10.1002/14651858.CD002098.pub2]

15 **Dixon RF**, Zisser H, Layne JE, Barleen NA, Miller DP, Moloney DP, Majithia AR, Gabbay RA, Riff J. A Virtual Type 2 Diabetes Clinic Using Continuous Glucose Monitoring and Endocrinology Visits. *J Diabetes Sci Technol* 2020; **14**: 908-911 [PMID: 31762302 DOI: 10.1177/1932296819888662]

16 **Fisher L**, Polonsky W, Asuni A, Jolly Y, Hessler D. The early impact of the COVID-19 pandemic on adults with type 1 or type 2 diabetes: A national cohort study. *J Diabetes Complications* 2020; **34**: 107748 [PMID: 33059981 DOI: 10.1016/j.jdiacomp.2020.107748]

17 **Bindom SM**, Lazartigues E. The sweeter side of ACE2: physiological evidence for a role in diabetes. *Mol Cell Endocrinol* 2009; **302**: 193-202 [PMID: 18948167 DOI: 10.1016/j.mce.2008.09.020]

18 **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]

19 **Li J**, Wang X, Chen J, Zuo X, Zhang H, Deng A. COVID-19 infection may cause ketosis and ketoacidosis. *Diabetes Obes Metab* 2020; **22**: 1935-1941 [PMID: 32314455 DOI: 10.1111/dom.14057]

20 **Kim NY**, Ha E, Moon JS, Lee YH, Choi EY. Acute Hyperglycemic Crises with Coronavirus Disease-19: Case Reports. *Diabetes Metab J* 2020; **44**: 349-353 [PMID: 32347027 DOI: 10.4093/dmj.2020.0091]

21 **Cassar MR**, Borg D, Camilleri L, Schembri A, Anastasi EA, Buhagiar K, Callus C, Grech M. A novel use of telemedicine during the COVID-19 pandemic. *Int J Infect Dis* 2021; **103**: 182-187 [PMID: 33246041 DOI: 10.1016/j.ijid.2020.11.170]

22 **Garg SK**, Rodbard D, Hirsch IB, Forlenza GP. Managing New-Onset Type 1 Diabetes During the COVID-19 Pandemic: Challenges and Opportunities. *Diabetes Technol Ther* 2020; **22**: 431-439

[PMID: 32302499 DOI: 10.1089/dia.2020.0161]

23 **Ohannessian R**, Duong TA, Odone A. Global Telemedicine Implementation and Integration Within Health Systems to Fight the COVID-19 Pandemic: A Call to Action. *JMIR Public Health Surveill* 2020; **6**: e18810 [PMID: 32238336 DOI: 10.2196/18810]

24 **Zimmer-Galler IE**, Kimura AE, Gupta S. Diabetic retinopathy screening and the use of telemedicine. *Curr Opin Ophthalmol* 2015; **26**: 167-172 [PMID: 25759962 DOI: 10.1097/ICU.0000000000000142]

25 **Monaghesh E**, Hajizadeh A. The role of telehealth during COVID-19 outbreak: a systematic review based on current evidence. *BMC Public Health* 2020; **20**: 1193 [PMID: 32738884 DOI: 10.1186/s12889-020-09301-4]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 786-793

DOI: 10.4239/wjd.v12.i6.786

ISSN 1948-9358 (online)

MINIREVIEWS

Oral glucose tolerance test in diabetes, the old method revisited

Feng Yu Kuo, Kai-Chun Cheng, Yingxiao Li, Juei-Tang Cheng

ORCID number: Feng Yu Kuo 0000-0002-9254-7753; Kai-Chun Cheng 0000-0003-4983-4666; Yingxiao Li 0000-0003-4858-8475; Juei-Tang Cheng 0000-0002-4522-584X.

Author contributions: Kuo FY and Cheng KC designed the article; Kuo FY and Li Y collected the references and prepared the manuscript; Cheng JT revised the manuscript for important intellectual content; all authors read and approved the final manuscript.

Conflict-of-interest statement: No conflict of interest.

Manuscript source: Invited manuscript

Specialty type: Methodology

Feng Yu Kuo, Cardiovascular Center, Veterans General Hospital, Kaohsiung 82445, Taiwan

Kai-Chun Cheng, Department of Pharmacy, College of Pharmacy and Health Care, Tajen University, Pingtung 90741, Taiwan

Kai-Chun Cheng, Pharmacological Department of Herbal Medicine and Department of Psychosomatic Internal Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8544, Japan

Yingxiao Li, Department of Nursing, Tzu Chi University of Science and Technology, Hualien 973302, Taiwan

Juei-Tang Cheng, Department of Medical Research, Chi-Mei Medical Center, Tainan 71004, Taiwan

Corresponding author: Juei-Tang Cheng, PhD, Additional Professor, Department of Medical Research, Chi-Mei Medical Center, No. 901 Zhonghua Road, Yong Kang District, Tainan 71004, Taiwan. jtcheng5503@gmail.com

Abstract

The oral glucose tolerance test (OGTT) has been widely used both in clinics and in basic research for a long time. It is applied to diagnose impaired glucose tolerance and/or type 2 diabetes mellitus in individuals. Additionally, it has been employed in research to investigate glucose utilization and insulin sensitivity in animals. The main aim of each was quite different, and the details are also somewhat varied. However, the time or duration of the OGTT was the same, using the 2-h post-glucose load glycemia in both, following the suggestions of the American Diabetes Association. Recently, the use of 30-min or 1-h post-glucose load glycemia in clinical practice has been recommended by several studies. In this review article, we describe this new view and suggest perspectives for the OGTT. Additionally, quantification of the glucose curve in basic research is also discussed. Unlike in clinical practice, the incremental area under the curve is not suitable for use in the studies involving animals receiving repeated treatments or chronic treatment. We discuss the potential mechanisms in detail. Moreover, variations between bench and bedside in the application of the OGTT are introduced. Finally, the newly identified method for the OGTT must achieve a recommendation from the American Diabetes Association or another official unit soon. In conclusion, we summarize the recent reports regarding the OGTT and add some of our own perspectives, including machine learning and others.

Key Words: Oral glucose tolerance test; Impaired glucose tolerance; Glucose Utilization;

Country/Territory of origin: Taiwan

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

Received: January 14, 2021
Peer-review started: January 14, 2021
First decision: February 12, 2021
Revised: February 24, 2021
Accepted: May 19, 2021
Article in press: May 19, 2021
Published online: June 15, 2021

P-Reviewer: Kim IJ, Momčilović S, Novita BD
S-Editor: Wang JL
L-Editor: Filipodia
P-Editor: Li JH

Type 2 diabetes; Area under the curve

Core Tip: Oral glucose tolerance test (OGTT) is a useful tool that has been applied from the last century to now. It is used to diagnose impaired glucose tolerance and/or type 2 diabetes mellitus in individuals. Basic research also applied it to investigate the glucose utilization and insulin sensitivity in animals. However, the main aim of each is quite different, and the details are also somewhat varied. In addition to the merits of OGTT in bench and bedside, variations between clinical practice and basic research are also discussed. Notably, recent reports have recommended that the time for OGTT be shorter in individuals. This conclusion needs to be confirmed officially in advance by diabetes associations. This new method is also required to be clarified in animal research. Additionally, perspectives of OGTT application are also conducted in this review including machine learning. Therefore, this report suggests a new way for OGTT practice in the future.

Citation: Kuo FY, Cheng KC, Li Y, Cheng JT. Oral glucose tolerance test in diabetes, the old method revisited. *World J Diabetes* 2021; 12(6): 786-793
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/786.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.786

INTRODUCTION

The oral glucose tolerance test (OGTT) has widely been used in clinics to diagnose impaired glucose tolerance (IGT) and/or type 2 diabetes mellitus (T2DM)[1]. The risk of transient postprandial hypoglycemia in patients with non-alcoholic fatty liver disease has also been identified using the OGTT[2]. Moreover, non-alcoholic steatohepatitis (NASH) linked with T2DM has been a focus, because NASH often occurs within 5 years in patients with T2DM (about 56.49%)[3]. Therefore, the application of OGTT for the diagnosis of non-alcoholic fatty liver disease or NASH is also popular in clinical practice.

The prevalence of T2DM is increasing at an alarming rate and is projected to increase from 171 million individuals in 2000 to 366 million by the year 2030[4]. In the United States, the number of adults living with T2DM is estimated to increase from 463.0 million to 700.2 million between 2019 and 2045. The total annual costs of managing this disease are expected to increase accordingly from 760.3 billion USD to 845.0 billion USD in this period. Therefore, the identification of IGT is important for T2DM prevention strategies in those who are at high risk. To achieve this, the OGTT has been suggested[5]. The use of glycated hemoglobin (HbA1c) levels has been proposed as an alternative to the OGTT. However, using only HbA1c to diagnose diabetes misses more than half of the diabetes cases established by the OGTT[6]. Therefore, the OGTT was introduced as the most suitable method[7].

The OGTT is also used in basic research, mainly focusing on glucose homeostasis of animals. Insulin resistance (IR) and insulin sensitivity have been identified using the results of the glucose- insulin index obtained from the OGTT in animals[8]. The diagnosis of T2DM was not included in this basic research. IGT in animals was also the main target in basic studies. Although research in animals may be useful to studying the basis of human disease, there are clear differences between species regarding metabolic regulation[9]. Therefore, the OGTT has limitations in basic research[10].

The OGTT has been applied over the last century by using the plasma glucose concentrations, measured after either an overnight fast or glucose loading, as a useful tool for diagnosing IGT. Indications for performing the OGTT are numerous, as described in a recent review article[11]. In this report, we explore the concerns regarding the OGTT, revisited for both bedside and bench.

OGTT IN CLINICAL PRACTICE

The OGTT was standardized by establishing an oral glucose load of 75 g and 2-h post-glucose load glycemia (2hPG), according to the Expert Committee of the American Diabetes Association (ADA)[12]. Overnight fasting glucose (FPG) and impaired fasting glycemia (IFG) were also recommended by the ADA. However, the FPG cut-off values for diabetes and/or IFG are far from being equivalent to the corresponding 2hPG values according to epidemiological data[13]. Additionally, it has been documented that impairment in insulin secretion is more relevant in IFG, while faltering insulin sensitivity is peculiar to IGT[14]. Otherwise, the concerns regarding the OGTT are that it is time consuming, poorly reproducible, and not well accepted by patients. Therefore, the ADA expected to include more subjects whose OGTT results were conclusive for diabetes or IGT, as described previously[15].

Although FPG cannot be equated to 2hPG, it has been demonstrated that the 2hPG predicts the risk of heart disease more effectively than FPG[16]. Basically, the plasma glucose levels obtained during the OGTT are related to both insulin sensitivity and secretion. As β-cell function is already substantially impaired in prediabetes, shortening the OGTT to use the 30-min or 1-h post-glucose load glycemia (1hPG) has recently been suggested[11]. Therefore, identifying high-risk individuals using the 1hPG seems an important and novel strategy to prevent the development of T2DM and cardiovascular disease. The addition of 30-min PG values to traditional glucose biomarker such as FPG and 2hPG values may assist the identification[11]. However, the faster the post-load glucose drops towards FPG, or the lower the rise in post-load glucose, the more efficient the β-cell function[15]. Another review article summarized the clinical reports to suggest that a 1hPG level of ≥ 8.6 mmol/L (or 155 mg/dL) to identify individuals with reduced β-cell function should be considered for adoption in clinical practice[17]. One-hour time points during a standard OGTT and the morphological characteristics of the glucose curve during the OGTT are associated with heightened risk of incident diabetes. The 30-min PG indicates first-phase insulin response. Diminution of the 30-min PG suggests β-cell dysfunction as an early lesion in the development of T2DM.

OGTT THROUGH QUANTITATIVE ANALYSIS

The shape of the glucose curve follows the pattern of a rise and fall in blood glucose after a fixed glucose loading, most commonly after a 2-h 75 g OGTT. The curve shape can be grouped into three categories by the blood glucose levels collected at fixed time points (such as 0, 15, 30, 60, 90, and 120 min) - monophasic (a gradual increase in glucose with a single peak and then a fall), biphasic (a gradual rise to a peak, a fall in glucose to a nadir and a subsequent rise), and unclassified (a continuous rise without a peak). The rationale for using these definitions is mainly due to the association of the curve shapes with pathological features of T2DM and the ease of categorization. The monophasic and unclassified curves, compared to the biphasic curve, are associated with lower insulin sensitivity and decreased β-cell function[18]. Additionally, the monophasic and unclassified curves are better predictors of prediabetes in individuals at high risk of diabetes[19]. However, the application of simple shape changes to diagnosing prediabetes and/or diabetes is challenging, as described recently[11]. A monophasic curve was identified during a 2-h test, but it became a biphasic curve after a 3-h test for no discernible reason[20].

Latent class trajectory analysis is another statistical tool that supplies probabilities for grouping pairs into different morphological classes while considering measurement error and intra-individual variability[21]. Four patterns have been described (Classes 1–4) that correspond to increasing glucose levels and declining insulin sensitivity and secretion with time[22]. However, concerns related to increased cost and patient burden associated with collecting blood at one to three additional time points and the expertise required to assess heterogeneity in curve shapes have limited its clinical use[23].

The area under the curve (AUC) is derived from the OGTT data to calculate the total rise in blood glucose during the OGTT using the trapezoidal rule[24]. It has been applied in scientific reports to show the variations in increased blood glucose during the OGTT. However, a marked difference in fasting blood glucose between individuals interrupted the data of the AUC. Therefore, the incremental AUC (iAUC) was developed to minimize this difference[25]. However, the iAUC obtained by subtracting the baseline value of fasting plasma glucose has been challenged as being

problematic[24]. Then, the positive incremental AUC (pAUC) was further suggested, and only the values above the baseline value were considered; those below the baseline were ignored in studies[25]. The total AUC (tAUC), iAUC, and positive incremental area under the curve (pAUC) have been applied in clinical practice. It has been indicated that the tAUC expresses the best correlation with the 2-h glucose level from the OGTT, and the total glucose response was better represented by the tAUC than by the iAUC or pAUC in a clinical report[26]. Mathematically, iAUC is suitably indicated by ΔAUC. However, ΔAUC has widely been applied in pharmacokinetics in another method. Therefore, iAUC is more popular than ΔAUC for applications in metabolic research. In epidemiological analysis, the superiority of the AUC for identifying individuals at high risk for progression to T2DM has been demonstrated [27]. However, application of the AUC in clinical practice is not popular[11].

Sophisticated mathematical and statistical methods such as machine learning algorithms have been developed to extract the features from OGTT glucose curves to predict diabetes[28]. Using a simplified, integrated model that is freely available online will increase the accessibility for OGTT analysis, as described previously[11].

OGTT IN BASIC RESEARCH

In basic research, the use of the OGTT in animals has mainly focused on glucose homeostasis. Unlike in clinical practice, the OGTT has not been used for diagnosis in basic research. IR and insulin sensitivity were identified using the results of the glucose- insulin index obtained from the OGTT in animals. Generally, IGT is widely reflected in a larger iAUC of the plasma glucose disappearance curve during the OGTT. The OGTT showed a marked increase in $AUC_{0\text{-}120min}$ from the experimental animals, indicating success in the induction of a diabetic model[8,29]. Diabetic animals were then used to screen the activity of an investigated substance, either a herbal extract or a nutrient. When the slope of the glucose disposal phase is markedly changed and the AUC is lower than that of the vehicle-treated control, it means that the investigated substance has the ability to alleviate IGT, probably due to enhanced glucose utilization[9]. Based on this merit, the AUC of OGTT data has been widely applied in animal research. The shape of the glucose curve during the OGTT is used as a reference only.

Generally, the animal subjects of these studies were maintained in a room under constant temperature and humidity, receiving standard chow. The FPG levels were stable without critical variations between animals, which is quite different from those of individuals in clinical practice. However, the FPG level can be affected by the use of agents in animals receiving a repeated daily treatment for several days; this has pharmacologically been termed as a “chronic effect”. Unlike in clinical practice, the changed FPG cannot be ignored, as described previously[10]. An agent, either a chemical compound or a natural product, may interrupt glucose homeostasis during chronic treatment[30]. Fortunately, no report has applied the iAUC in animals receiving such chronic treatment[8]. This means that researchers understand the situation regarding changes in glucose homeostasis induced by an agent during chronic treatment. Therefore, the AUC is generally used in all reports including samples that show a critical reduction in FPG after chronic treatment in diabetic animals.

Moreover, the plasma insulin level during the OGTT has also been a focus of basic research. Hyperglycemia may stimulate higher secretion of insulin to result in an increase in the plasma insulin level. Therefore, the shape of the insulin curve in parallel to that of the glucose curve may assist as a reference for the condition of insulin secretion and/or insulin sensitivity. However, it is difficult to assess changes in insulin potency in clinical practice, and there is a gap in the current scientific literature on insulin stability.

Overall, the OGTT in clinical practice is not the same as that used in basic research, as shown in Table 1. However, the merits of the OGTT for diagnostic use in clinics and for screening activity in basic research have been applied for many years[11]. The glucose curve supplies a brief indication of insulin sensitivity and secretion on the blood glucose level after a fixed glucose load. A 2-h 75 g OGTT is widely applied in clinical practice, and the same has also been applied in basic research, except the loaded glucose amount was modified. When the OGTT is revised to 30-min or 1hPG in clinical practice, the protocol of the OGTT in basic research should also be improved.

Table 1 Differences in the oral glucose tolerance test used in clinics and in basic research

Subjects	Clinical Practice	Basic Research
Main aims	Diagnosis	Assay of responses
Applications	75 g for 2hPG	2 g for 2hPG
New method	75 g for 30 min or1hPG	Unknown
Identification	Shape of curve	Calculated AUC
Fasting PG	Important	Included
Plasma insulin	Reference	Important
Conscious	Clear	Anesthesia
Cost-effective	No	Yes
Interpretation	Diet and exercise	Pain sensation
Circadian factor	Yes	Can be regulated
Bias	Allergy to glucose	Artificial errors
Fasting concerns	Yes	No
Reproducibility	Not so good	Reliable
Drug interaction	Yes	No
Indications	Anemia or borderline PG	Less
Others	Age or renal glycosuria	Genetics

1hPG: 1-h post-glucose load glycemia; 2hPG: 2-h post-glucose load glycemia; AUC: Area under the curve; PG: Post-glucose load glycemia.

OGTT IN PERSPECTIVE

The FPG, 2hPG, and HbA1c have been indicated to have performance limitations that seem to make them unsuitable for the diagnosis of high-risk individuals[11]. An alternative method is consequently required. Therefore, a 30-min or 1hPG OGTT has been suggested, using a level of ≥ 8.6 mmol/L (or 155 mg/dL) as the criterion in clinical practice[17]. Recently, diabetes prediction models using the OGTT with or without other metabolic risk factors have been reported. A historical cohort study compared the future risk for diabetes among groups using the insulinogenic index [31]. The time to glucose peak could be a valuable epidemiological tool to indicate β-cell function in populations with a high risk of diabetes[32].

New biomarkers in circulation after glucose loading are also helpful in the diagnosis of T2DM. Fasting is important to the assay but is not favored by the individuals who received the OGTT. Therefore, circulating biomarkers less influenced by food and/or feeding are more useful. These biomarkers remain to be found and developed in the future. It has been demonstrated that the output of incretins, including glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide, is negatively associated with higher IR biomarkers, such as: HOMA-IR, fasting insulin, and fasting free fatty acid levels[33]. However, endogenous incretins are regulated by glycemia, particularly intestinal glucose[34]. Therefore, incretins seem unsuitable for use as biomarkers in clinical practice. Otherwise, delay in the glucose peak time in individuals shows a gradual aggravation in glucose metabolism and a decrease in insulin sensitivity and/or secretion[35]. However, the peak and decline in plasma glucose levels during the OGTT reflect the interplay between multiple factors. Thus, application of the OGTT seems limited in the study of the pathogenesis of T2DM without other indicators as described above.

Reactive hypoglycemia (RH) has been mentioned in clinical practice, probably due to gastrointestinal dysfunction or insufficiency that leads to relative insulin secretion or increased insulin sensitivity[36]. Obese individuals have higher rates of RH after a prolonged OGTT in clinics. Hypoglycemia may be due to a variety of reasons, such as increased endogenous insulin, low secretion of anti-insulin hormones, or organic lesions such as insulinoma, proliferation of islet β cells, or drug-induced hypoglycemia caused by overtreatment in patients with diabetes[37]. Biomarkers involved in RH remain obscure and could be a good target to develop.

Osteocalcin levels are negatively associated with glucose[38]. People with diabetes have lower levels of osteocalcin, higher levels of glucose, and lower levels of insulin when fasting. During the OGTT, both bone resorption markers and bone formation markers decrease within 20 min[39], although insulin does not increase osteoblastic production of osteocalcin in healthy humans. Therefore, endogenous substances regulated with glucose homeostasis may be suitable for development as biomarkers.

Machine learning has been reported to be capable of predicting glucose tolerance [40]. A support vector machine along with a rule-based explanation was documented for extracting features from OGTT data for the prediction of diabetes[28]. The features deduced from the plasma glucose concentrations provide the optimal feature subset and have the strongest predictive power for the future development of T2DM. This may provide a complementary and cost-effective tool for clinicians to screen outcomes. Moreover, the prediction of IGT *via* machine learning could also be employed to fill in IGT status when the OGTT is technically not possible or to estimate retroactively IGT status from stored fasting samples[40]. Due to this minimization of the limitations, machine learning is helpful in clinical practice.

CONCLUSION

There is no doubt that the OGTT is a useful tool; it has been applied since 1885, when it was proposed. It will continue to be used in the future with mild improvements, made by step by step. It has been widely suggested in recent years that the duration of the OGTT should be shortened to use the 30-min or 1hPG. The glucose level obtained from a single OGTT could be a valuable tool of high clinical significance and could enhance prediabetes risk stratification. The derived problem, including the calculation of the AUC, shall be a concern in the future. Basic research has also applied this tool with different aims. It is still uncertain whether or not a shorter version of the OGTT is suitable for animals. Altogether, the OGTT will be able to be applied continuously from bench to bedside without hesitation once each problem has been addressed.

REFERENCES

1 **Guariguata L**, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014; **103**: 137-149 [PMID: 24630390 DOI: 10.1016/j.diabres.2013.11.002]

2 **Adam JM**, Josten D. Isolated post-challenge hyperglycemia: concept and clinical significance. *Acta Med Indones* 2008; **40**: 171-175 [PMID: 18838757]

3 **Morio R**, Hyogo H, Hatooka M, Morio K, Kan H, Kobayashi T, Kawaoka T, Tsuge M, Hiramatsu A, Imamura M, Kawakami Y, Aikata H, Ochi H, Masayasu Y, Chayama K. The risk of transient postprandial oxyhypoglycemia in nonalcoholic fatty liver disease. *J Gastroenterol* 2017; **52**: 253-262 [PMID: 27351871 DOI: 10.1007/s00535-016-1236-7]

4 **Seetlani NK**, Memon AR, Tanveer S, Ali A, Ali P, Imran K, Haroon H. Frequency of Non-Alcoholic Steatohepatitis on Histopathology in Patients of Type 2 Diabetes Mellitus with Duration of More than 5 Years. *J Coll Physicians Surg Pak* 2016; **26**: 643-646 [PMID: 27539754]

5 **Wild S**, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]

6 **Perreault L**, Kahn SE, Christophi CA, Knowler WC, Hamman RF; Diabetes Prevention Program Research Group. Regression from pre-diabetes to normal glucose regulation in the diabetes prevention program. *Diabetes Care* 2009; **32**: 1583-1588 [PMID: 19587364 DOI: 10.2337/dc09-0523]

7 **Peter A**, Fritsche A, Stefan N, Heni M, Häring HU, Schleicher E. Diagnostic value of hemoglobin A1c for type 2 diabetes mellitus in a population at risk. *Exp Clin Endocrinol Diabetes* 2011; **119**: 234-237 [PMID: 21264802 DOI: 10.1055/s-0030-1270440]

8 **Babbar R**, Heni M, Peter A, Hrabě de Angelis M, Häring HU, Fritsche A, Preissl H, Schölkopf B, Wagner R. Prediction of Glucose Tolerance without an Oral Glucose Tolerance Test. *Front Endocrinol (Lausanne)* 2018; **9**: 82 [PMID: 29615972 DOI: 10.3389/fendo.2018.00082]

9 **Kuo SC**, Li Y, Cheng JT. Glucose Tolerance Test Applied in Screening of Anti-Diabetic Agent(S). *Curre Res Diabetes Obes J* 2018; **7**: 555716 [DOI: 10.19080/CRDOJ.2018.07.555716]

10 **Kowalski GM**, Bruce CR. The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents. *Am J Physiol Endocrinol Metab* 2014; **307**: E859-E871 [PMID: 25205823 DOI: 10.1152/ajpendo.00165.2014]

11 **Cheng KC**, Li Y, Cheng JT. Limitations of Oral Glucose Tolerance Test in Animal Studies. *J Diabetes Treat* 2018; JDBT-146 [DOI: 10.29011/2574-7568.000046]

12 **Jagannathan R**, Neves JS, Dorcely B, Chung ST, Tamura K, Rhee M, Bergman M. The Oral

Glucose Tolerance Test: 100 Years Later. *Diabetes Metab Syndr Obes* 2020; **13**: 3787-3805 [PMID: 33116727 DOI: 10.2147/DMSO.S246062]
13 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997; **20**: 1183-1197 [PMID: 9203460 DOI: 10.2337/diacare.20.7.1183]
14 Will new diagnostic criteria for diabetes mellitus change phenotype of patients with diabetes? *BMJ* 1998; **317**: 371-375 [PMID: 9694750 DOI: 10.1136/bmj.317.7155.371]
15 **Carnevale Schianca GP**, Rossi A, Sainaghi PP, Maduli E, Bartoli E. The significance of impaired fasting glucose versus impaired glucose tolerance: importance of insulin secretion and resistance. *Diabetes Care* 2003; **26**: 1333-1337 [PMID: 12716784 DOI: 10.2337/diacare.26.5.1333]
16 **Bartoli E**, Fra GP, Carnevale Schianca GP. The oral glucose tolerance test (OGTT) revisited. *Eur J Intern Med* 2011; **22**: 8-12 [PMID: 21238885 DOI: 10.1016/j.ejim.2010.07.008]
17 **Perry RC**, Baron AD. Impaired glucose tolerance. Why is it not a disease? *Diabetes Care* 1999; **22**: 883-885 [PMID: 10372235 DOI: 10.2337/diacare.22.6.883]
18 **Bergman M**, Manco M, Sesti G, Dankner R, Pareek M, Jagannathan R, Chetrit A, Abdul-Ghani M, Buysschaert M, Olsen MH, Nilsson PM, Medina JL, Roth J, Groop L, Del Prato S, Raz I, Ceriello A. Petition to replace current OGTT criteria for diagnosing prediabetes with the 1-hour post-load plasma glucose ≥ 155 mg/dl (8.6 mmol/L). *Diabetes Res Clin Pract* 2018; **146**: 18-33 [PMID: 30273707 DOI: 10.1016/j.diabres.2018.09.017]
19 **Kaga H**, Tamura Y, Takeno K, Kakehi S, Someya Y, Funayama T, Furukawa Y, Suzuki R, Sugimoto D, Kadowaki S, Nishitani-Yokoyama M, Shimada K, Daida H, Aoki S, Giacca A, Sato H, Kawamori R, Watada H. Shape of the glucose response curve during an oral glucose tolerance test is associated with insulin clearance and muscle insulin sensitivity in healthy non-obese men. *J Diabetes Investig* 2020; **11**: 874-877 [PMID: 32020726 DOI: 10.1111/jdi.13227]
20 **Abdul-Ghani MA**, Lyssenko V, Tuomi T, Defronzo RA, Groop L. The shape of plasma glucose concentration curve during OGTT predicts future risk of type 2 diabetes. *Diabetes Metab Res Rev* 2010; **26**: 280-286 [PMID: 20503260 DOI: 10.1002/dmrr.1084]
21 **Tura A**, Morbiducci U, Sbrignadello S, Winhofer Y, Pacini G, Kautzky-Willer A. Shape of glucose, insulin, C-peptide curves during a 3-h oral glucose tolerance test: any relationship with the degree of glucose tolerance? *Am J Physiol Regul Integr Comp Physiol* 2011; **300**: R941-R948 [PMID: 21248305 DOI: 10.1152/ajpregu.00650.2010]
22 **Hulman A**, Simmons RK, Vistisen D, Tabák AG, Dekker JM, Alssema M, Rutters F, Koopman AD, Solomon TP, Kirwan JP, Hansen T, Jonsson A, Gjesing AP, Eiberg H, Astrup A, Pedersen O, Sørensen TI, Witte DR, Færch K. Heterogeneity in glucose response curves during an oral glucose tolerance test and associated cardiometabolic risk. *Endocrine* 2017; **55**: 427-434 [PMID: 27699707 DOI: 10.1007/s12020-016-1126-z]
23 **Hulman A**, Witte DR, Vistisen D, Balkau B, Dekker JM, Herder C, Hatunic M, Konrad T, Færch K, Manco M. Pathophysiological Characteristics Underlying Different Glucose Response Curves: A Latent Class Trajectory Analysis From the Prospective EGIR-RISC Study. *Diabetes Care* 2018; **41**: 1740-1748 [PMID: 29853473 DOI: 10.2337/dc18-0279]
24 **Hulman A**, Wagner R, Vistisen D, Færch K, Balkau B, Manco M, Golay A, Häring HU, Heni M, Fritsche A, Witte DR. Glucose Measurements at Various Time Points During the OGTT and Their Role in Capturing Glucose Response Patterns. *Diabetes Care* 2019; **42**: e56-e57 [PMID: 30692243 DOI: 10.2337/dc18-2397]
25 **Allison DB**, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer FX. The use of areas under curves in diabetes research. *Diabetes Care* 1995; **18**: 245-250 [PMID: 7729306 DOI: 10.2337/diacare.18.2.245]
26 **Cheng KC**, Li Y, Cheng JT. The Areas Under Curves (AUC) used in diabetes research: Update view. *Integr Obesity Diabetes* 2018; **4**: 1-2 [DOI: 10.15761/IOD.1000212]
27 **Khan A**, Hornemann T. Correlation of the plasma sphingoid base profile with results from oral glucose tolerance tests in gestational diabetes mellitus. *EXCLI J* 2017; **16**: 497-509 [PMID: 28694753 DOI: 10.17179/excli2017-171]
28 **Alyass A**, Almgren P, Akerlund M, Dushoff J, Isomaa B, Nilsson P, Tuomi T, Lyssenko V, Groop L, Meyre D. Modelling of OGTT curve identifies 1 h plasma glucose level as a strong predictor of incident type 2 diabetes: results from two prospective cohorts. *Diabetologia* 2015; **58**: 87-97 [PMID: 25292440 DOI: 10.1007/s00125-014-3390-x]
29 **Abbas HT**, Alic L, Erraguntla M, Ji JX, Abdul-Ghani M, Abbasi QH, Qaraqe MK. Predicting long-term type 2 diabetes with support vector machine using oral glucose tolerance test. *PLoS One* 2019; **14**: e0219636 [PMID: 31826018 DOI: 10.1371/journal.pone.0219636]
30 **Song P**, Kim JH, Ghim J, Yoon JH, Lee A, Kwon Y, Hyun H, Moon HY, Choi HS, Berggren PO, Suh PG, Ryu SH. Emodin regulates glucose utilization by activating AMP-activated protein kinase. *J Biol Chem* 2013; **288**: 5732-5742 [PMID: 23303186 DOI: 10.1074/jbc.M112.441477]
31 **Aono D**, Oka R, Kometani M, Takeda Y, Karashima S, Yoshimura K, Yoneda T. Insulin Secretion and Risk for Future Diabetes in Subjects with a Nonpositive Insulinogenic Index. *J Diabetes Res* 2018; **2018**: 5107589 [PMID: 29765987 DOI: 10.1155/2018/5107589]
32 **Chung ST**, Ha J, Onuzuruike AU, Kasturi K, Galvan-De La Cruz M, Bingham BA, Baker RL, Utumatwishima JN, Mabundo LS, Ricks M, Sherman AS, Sumner AE. Time to glucose peak during an oral glucose tolerance test identifies prediabetes risk. *Clin Endocrinol (Oxf)* 2017; **87**: 484-491 [PMID: 28681942 DOI: 10.1111/cen.13416]
33 **Liu KF**, Niu CS, Tsai CJ, Yang LG, Peng WH, Niu HS. Comparison of area under the curve in

various models of diabetic rats receiving chronic medication. *Arch Med Sci* 2020 [DOI: 10.5114/aoms.2019.91471]

34 **Kiec-Klimczak M**, Malczewska-Malec M, Razny U, Zdzienicka A, Gruca A, Goralska J, Pach D, Gilis-Januszewska A, Dembinska-Kiec A, Hubalewska-Dydejczyk A. Assessment of incretins in oral glucose and lipid tolerance tests may be indicative in the diagnosis of metabolic syndrome aggravation. *J Physiol Pharmacol* 2016; **67**: 217-226 [PMID: 27226181]

35 **Shen J**, Chen Z, Chen C, Zhu X, Han Y. Impact of incretin on early-phase insulin secretion and glucose excursion. *Endocrine* 2013; **44**: 403-410 [PMID: 23283820 DOI: 10.1007/s12020-012-9867-9]

36 **Kim JY**, Michaliszyn SF, Nasr A, Lee S, Tfayli H, Hannon T, Hughan KS, Bacha F, Arslanian S. The Shape of the Glucose Response Curve During an Oral Glucose Tolerance Test Heralds Biomarkers of Type 2 Diabetes Risk in Obese Youth. *Diabetes Care* 2016; **39**: 1431-1439 [PMID: 27293201 DOI: 10.2337/dc16-0352]

37 **Lv X**, Fang K, Hao W, Han Y, Yang N, Yu Q. Identification of Reactive Hypoglycemia with Different Basic BMI and Its Causes by Prolonged Oral Glucose Tolerance Test. *Diabetes Metab Syndr Obes* 2020; **13**: 4717-4726 [PMID: 33293845 DOI: 10.2147/DMSO.S280084]

38 **Douillard C**, Jannin A, Vantyghem MC. Rare causes of hypoglycemia in adults. *Ann Endocrinol (Paris)* 2020; **81**: 110-117 [PMID: 32409005 DOI: 10.1016/j.ando.2020.04.003]

39 **Starup-Linde J**, Lykkeboe S, Gregersen S, Hauge EM, Langdahl BL, Handberg A, Vestergaard P. Differences in biochemical bone markers by diabetes type and the impact of glucose. *Bone* 2016; **83**: 149-155 [PMID: 26555635 DOI: 10.1016/j.bone.2015.11.004]

40 **Starup-Linde J**, Westberg-Rasmussen S, Lykkeboe S, Handberg A, Hartmann B, Holst JJ, Hermansen K, Vestergaard P, Gregersen S. Glucose Tolerance Tests and Osteocalcin Responses in Healthy People. *Front Endocrinol (Lausanne)* 2018; **9**: 356 [PMID: 30057568 DOI: 10.3389/fendo.2018.00356]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 794-809

DOI: 10.4239/wjd.v12.i6.794

ISSN 1948-9358 (online)

MINIREVIEWS

Diabetic gastroenteropathy: An underdiagnosed complication

Marcio José Concepción Zavaleta, Jhean Gabriel Gonzáles Yovera, Diego Martín Moreno Marreros, Luciana del Pilar Rafael Robles, Kely Roxana Palomino Taype, Karen Nohelly Soto Gálvez, Luis Fernando Arriola Torres, Julia Cristina Coronado Arroyo, Luis Alberto Concepción Urteaga

ORCID number: Marcio José Concepción Zavaleta 0000-0001-9719-1875; Jhean Gabriel Gonzáles Yovera 0000-0002-5809-3006; Diego Martín Moreno Marreros 0000-0001-5396-7360; Luciana del Pilar Rafael Robles 0000-0003-2276-1575; Kely Roxana Palomino Taype 0000-0002-7172-6138; Karen Nohelly Soto Gálvez 0000-0003-3437-3617; Luis Fernando Arriola Torres 0000-0001-5365-9800; Julia Cristina Coronado Arroyo 0000-0002-4916-8205; Luis Alberto Concepción Urteaga 0000-0003-0462-3101.

Author contributions: Concepción Zavaleta MJ and Gonzáles Yovera JG were the main writer and performed literature overview; Moreno Marreros DM was reviewer, translator, and prepared the manuscript; Rafael Robles LP conceived and designed the study, and was reviewer; Palomino Taype KR, Soto Galvez KN and Arriola Torres LF performed written contribution to body of text; Coronado Arroyo JC and Concepción Zavaleta LA performed literature overview and were reviewers; All authors have read and approve the final manuscript.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Marcio José Concepción Zavaleta, Division of Endocrinology, Hospital Nacional Guillermo Almenara Irigoyen, Lima 12590, Peru

Jhean Gabriel Gonzáles Yovera, Division of Gastroenterology, Hospital Nacional Guillermo Almenara Irigoyen, Lima 12590, Peru

Diego Martín Moreno Marreros, Division of Medicine, Universidad Nacional de Trujillo, Trujillo 13011, Peru

Luciana del Pilar Rafael Robles, Division of Endocrinology, Hospital Nacional Dos de Mayo, Lima 150101, Peru

Kely Roxana Palomino Taype, Division of Endocrinology, Instituto de Neurología y Endocrinología Avanzada, Andahuaylas 03700, Peru

Karen Nohelly Soto Gálvez, Clinical Management Unit, Hospital Nacional Guillermo Almenara Irigoyen, Lima 12590, Peru

Luis Fernando Arriola Torres, Division of Neurology, Instituto de Neurología y Endocrinología Avanzada, Andahuaylas 03700, Peru

Julia Cristina Coronado Arroyo, Division of Obstetrics and Gynaecology, Clínica Vesalio, Lima 15036, Peru

Luis Alberto Concepción Urteaga, Division of Neumology, Hospital Regional Docente de Trujillo, Trujillo 13011, Peru

Corresponding author: Diego Martín Moreno Marreros, MD, Research Associate, Division of Medicine, Universidad Nacional de Trujillo, Fco. Adrianzen 312 Urb. Santa María, Trujillo 13011, Peru. diegomorenosud@hotmail.com

Abstract

This article is an extensive review that provides an update on the pathophysiology, symptoms, diagnosis, and treatment of diabetic gastroenteropathy. There is no reported prevalence, but it has been described that patients with type 1 diabetes have a cumulative incidence at 10 years of 5.2%, and type 2 patients, 1%. Also, in the group of type 1 diabetes, it has been observed that women are more likely to present this condition (5.8% *vs* 3.5%). Many factors are associate with its development (*e.g.*, hyperglycemia, vagal dysfunction, loss of expression of neural

Manuscript source: Invited manuscript

Specialty type: Endocrinology and Metabolism

Country/Territory of origin: Peru

Peer-review report's scientific quality classification

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

Received: January 14, 2021
Peer-review started: January 14, 2021
First decision: February 12, 2021
Revised: February 28, 2021
Accepted: May 27, 2021
Article in press: May 27, 2021
Published online: June 15, 2021

P-Reviewer: Aureliano M, Zhang DM
S-Editor: Ma YJ
L-Editor: A
P-Editor: Ma YJ

nitric oxide synthase in the myenteric plexus, alterations in the Cajal interstitial cell network, and oxidative stress). Gastrointestinal discomfort could be perceived 70% higher in diabetic patients, describing that 25% of diabetic patients experience gastrointestinal symptoms. Diabetic enteropathy could affect any portion of the gastrointestinal tract, but esophageal alterations were described in more than 60% of diabetic patients, also 60% of them present constipation, and 20%, diarrhea. Gastric emptying scintigraphy is useful to evaluate gastroparesis, therefore, gastric retention of more than 60% at 2 h has a sensitivity of 100% and specificity of 20% for diagnosis; however, other studies such as breath tests, with a sensitivity of 89% and a specificity of 80%, or the endoscopic capsule contribute to the diagnosis. There is no cure; however, management must be multidisciplinary, focused on slowing the progression of diabetic gastroenteropathy, reducing symptoms, and restoring function; that includes nutritional recommendation, maintain glucose levels kept below 180 mg/dL, use of prokinetics, anti-emetics; nowadays, it has been special interest in surgical treatment, such as pyloroplasty, also gastric electrical stimulation appears to be another alternative.

Key Words: Gastroenteropathy; Gastroparesis; Diabetes mellitus; Complication; Gastrointestinal disease; Treatment

Core Tip: Diabetic gastroenteropathy is a common complication, poorly diagnosed in patients with long-term disease. These can present esophageal, gastric, intestinal, and even anorectal symptoms. Gastrointestinal Symptom Severity Index, Gastroparesis Cardinal Symptom Index, and Assessment of Constipation Quality of Life scores, as well as symptomatic assessment scales, contribute to the diagnosis. Apart from gastric emptying scintigraphy, currently, the use of endoscopic capsules has allowed the evaluation of abnormal transit. Jejunal fluid aspiration and culture allow assessment of bacterial overgrowth. Medical treatment, as well as adequate glycemic control, improve the symptoms, and delay the progression of the disease; in selected patients, pyloroplasty and gastric electrical stimulation are useful.

Citation: Concepción Zavaleta MJ, Gonzáles Yovera JG, Moreno Marreros DM, Rafael Robles LDP, Palomino Taype KR, Soto Gálvez KN, Arriola Torres LF, Coronado Arroyo JC, Concepción Urteaga LA. Diabetic gastroenteropathy: An underdiagnosed complication. *World J Diabetes* 2021; 12(6): 794-809
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/794.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.794

INTRODUCTION

Diabetic gastroparesis was first described in 1945 by Wayne Rundells, later in 1958, Kassander coined the term gastroparesis diabeticorum[1]. This is one of the most common complications in patients with long-term type 1 and type 2 diabetes mellitus, therefore almost one quarter of this population describes gastrointestinal symptoms; manifesting itself especially in those who evolve with inadequate glycemic control or with other complications at the same time. It is mainly characterized by presenting early satiety, prolonged postprandial fullness, abdominal distention, nausea and vomiting, and abdominal pain[2]. The pathophysiology of diabetic gastroenteropathy is complex. It is postulated that the enteric nervous system neuropathy induced by hyperglycemia is one of the main causes. There is also a loss of Cajal's interstitial cells and enteric glial cells. On the other hand, oxidative stress and inflammation also affect regenerative processes and signaling[3]. As part of the treatment in some patients is used insulin, which in this 2020 are 100 years since it was discover (see Figure 1)[4,5], and around 40 years before the term gastroparesis diabeticorum was coined.

Even though, this diabetic complication has been described five decades ago, diagnosis and management still represent a challenge for the physicians. The present

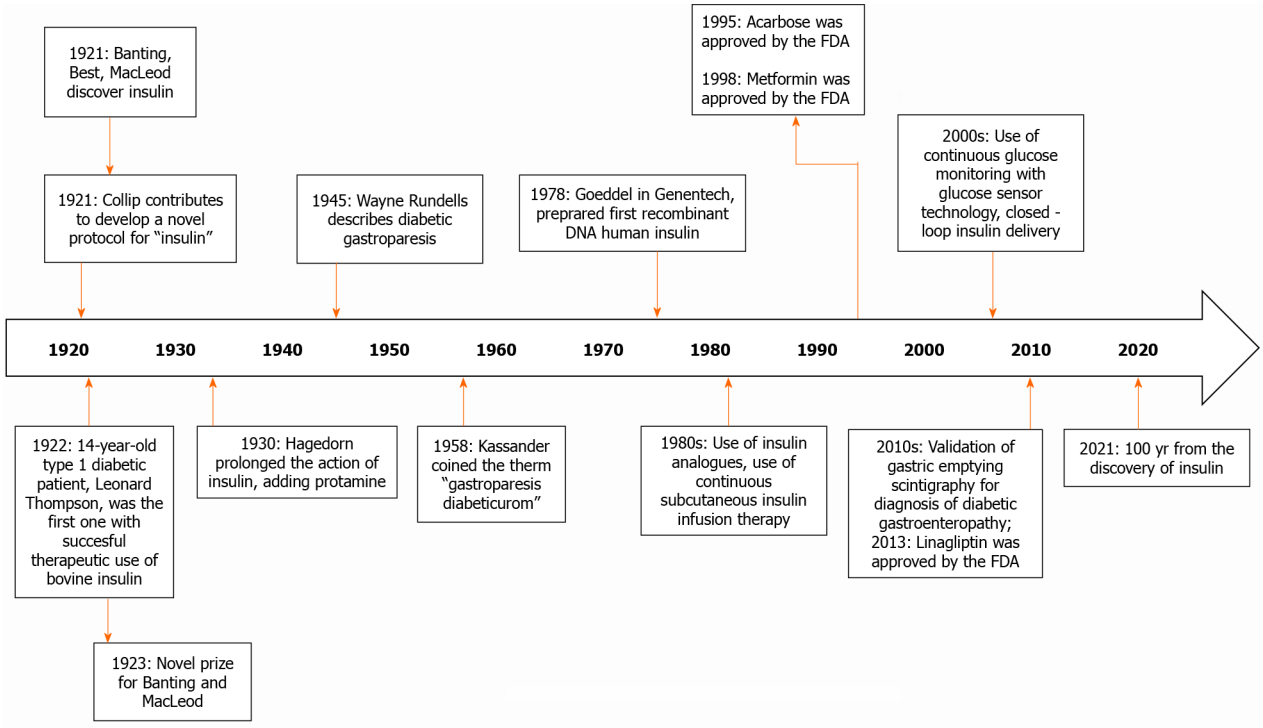

Figure 1 Timeline. Since the discovery of insulin until nowadays[4,5].

review focuses on the pathophysiology development, clinical, diagnosis, and brings new data about current and future treatment of this pathology. The aim of this review is an update on different treatments, including, changes in lifestyle, medical, and also surgical.

EPIDEMIOLOGY

Approximately 463 million people live with diabetes worldwide, and 32 million residing in South and Central America[6].

The prevalence of diabetic gastroenteropathy has not been established. Some reports mentioned a cumulative incidence of diabetic gastroparesis at 10 years of 5.2% for patients with type 1 diabetes mellitus and 1% for patients with type 2 diabetes mellitus [7,8]. However, due to the continuous increase in the number of patients with type 2 diabetes, these diabetic patients would represent a bigger group with this complication [9].

Regarding the symptoms, the prevalence of esophageal dysmotility is 63%, reflux is 41%, 60% of patients present constipation, and 20% manifest diarrhea[10]. These differences in prevalence probably respond to the lack of recognition of the signs and symptoms of gastroenteropathy, making it necessary to learn to recognize and treat them in time.

PHYSIOLOGY

Gastric emptying

Gastric emptying is a physiological process that allows the transit of digested food to the duodenum[2]. It is a mechanical process and is regulated by a complex neurohormonal control[11,12]. In neuronal control, the parasympathetic participates through the vagus nerve which afferences fibers arrive from the enteric system to the nucleus of the solitary tract, and then pass to the vagus dorsal motor nucleus in the spinal cord, from where it exits to the myenteric plexus (see Figure 2). In the gastric wall forming two vague motor circuits: Excitatory and inhibitory, which are distributed in a heterogeneous way (allowing an integrated function in gastric biomechanics) in the body, antrum, pylorus, and into the interstitial cells of the Cajal [11]. Additional, there are several neurotransmitters, that participate in neurohumoral

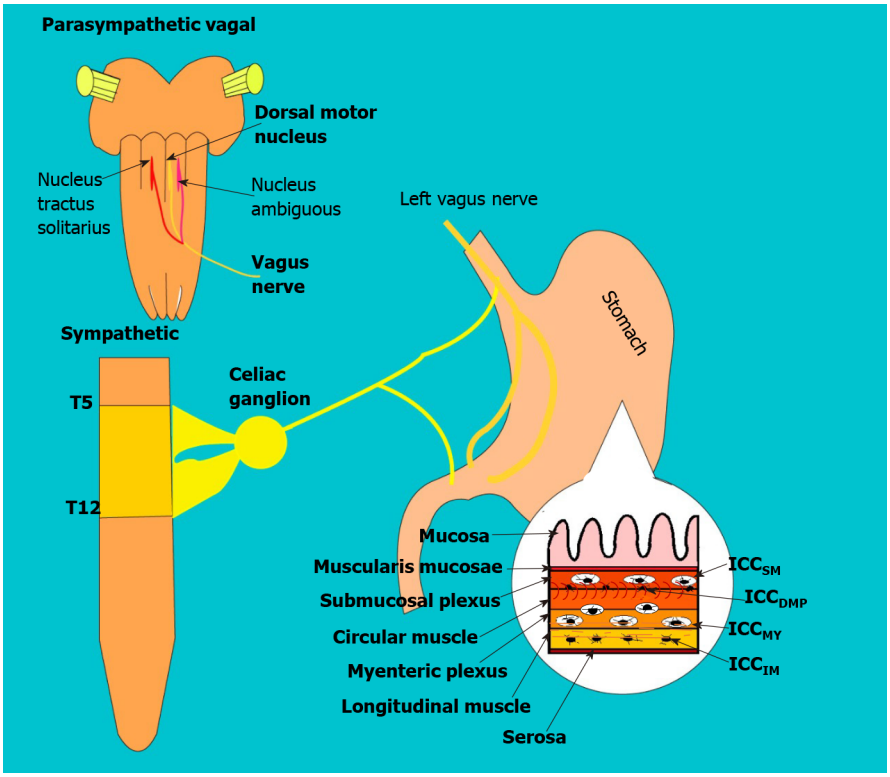

Figure 2 Control of gut motor function. The motor function of the gut is controlled by parasympathetic and sympathetic nervous systems, enteric neurons and interstitial cell of Cajal, smooth muscle cells. ICC: Interstitial cell of Cajal; ICC$_{SM}$: ICC-submucosal; ICC$_{DMP}$: ICC-deep muscular plexus; ICC$_{MY}$: ICC-myenteric; ICC$_{IM}$: ICC-intramuscular.

control at different levels (*e.g.*, Acetylcholine, Noradrenaline, GABA, dopamine, *etc.*), and hormones produced in the pancreas, stomach, small intestine, and in the nervous system (Some delay gastric emptying such as cholecystokinin, GLP- 1, and leptin; and others accelerate it such as motilin and ghrelin)[11].

The speed of emptying occurs differently according to the consistency and nature of the food, thus low-calorie liquids leave the stomach very quickly[11-13]. The most solid foods stay between 2 to 3 h in the stomach (because they are transformed into smaller particles to form the acid chyme and pass into the duodenum at an average speed of 1-4 Kcal/min)[14]. However, emptying is not always complete and exists an interdigestive period in which food particles pass without been digested. Recently, with advances in non-invasive studies, such as scintigraphy, the physiology of gastric emptying has been better understood[11]. The adequate gastric emptying biomechanics is the result of adequate coordination between the proximal and distal regions of the stomach[13]. The gastric fundus serves as a reservoir of content (initially, it relaxes due to nitrogen stimuli)[2,15] and then acts as a pressure pump. In the gastric body-antrum, the food content is mixed at a speed of 3 peristaltic waves per minute[12,13,16], the pylorus acts as a gate that filters only the 1-2 mm digested food and returns the largest foods[17]. In gastric emptying, 2 phases are recognized; the digestive (whose purpose is to transport the chyme to the duodenum) and the interdigestive (which several trains of peristaltic waves have the purpose of complete the gastric emptying of the indigestible particles); the hormones that accelerate emptying participate in this latter process[11].

PATHOPHYSIOLOGY

Gastroparesis is defined as delayed gastric emptying in the absence of mechanical obstruction. Although the exact mechanism of gastric dysfunction and the generation of symptoms are unknown, there are factors that promote its development, such as hyperglycemia, vagal dysfunction, loss of expression of neural nitric oxide synthase (nNOS) in the myenteric plexus, abnormalities of the Cajal interstitial cell network (ICC) and oxidative stress[17] (see Figure 3).

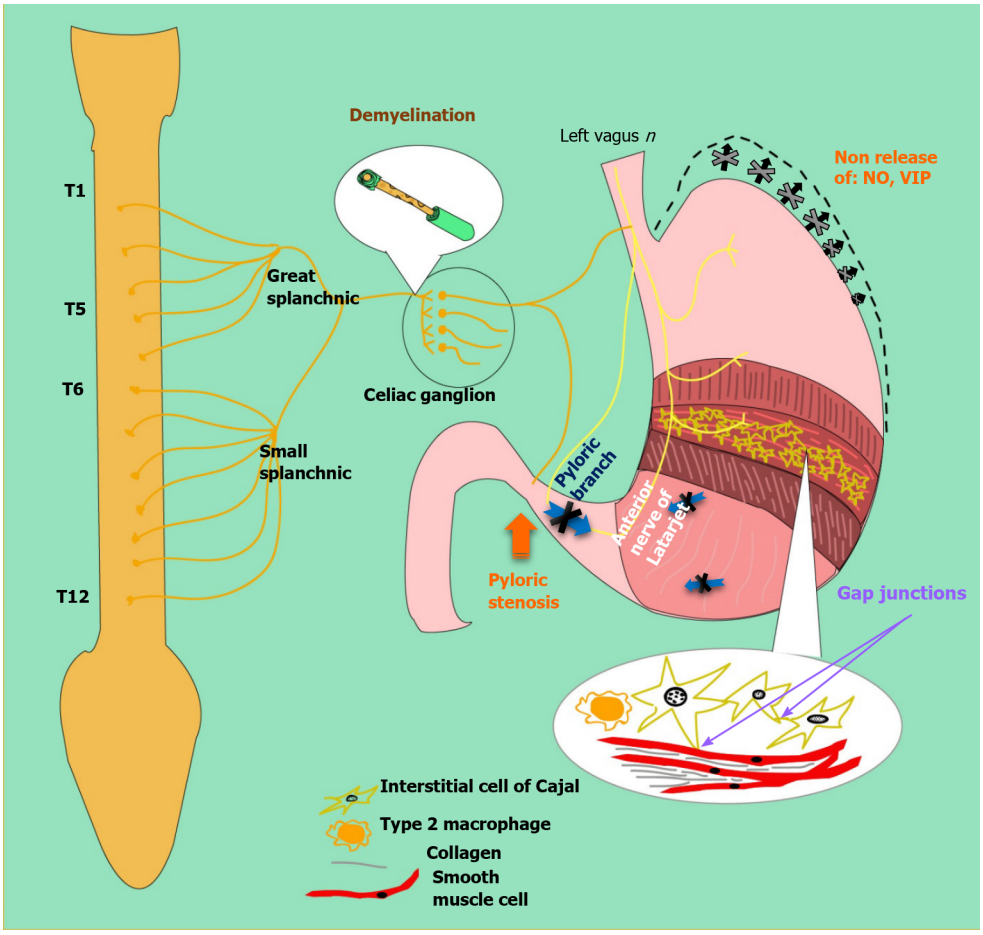

Figure 3 Pathophysiology of diabetic gastroparesis. Dysfunction in the Sympathetic nervous system (Celiac Ganglion), parasympathetic nervous system (Vagus Nerve) and enteric nervous system (Myenteric Interstitial cells of Cajal). Results in the dysfunction of emptying mechanism of the antral pump: phase of propulsion, emptying, and retropulsion (blue arrows), decrease of relaxes to accommodate meal (black arrows), loss of Nitric Oxide synthase in the enteric nerves (fundus, pylori), loss of ICC, Type 2 Macrophage altered function with loss of cytoprotective to ICC, smooth muscle atrophy, and increased smooth muscle fibrosis. NO: Nitric oxide; VIP: Vasoactive inhibitory peptide; ICC: Interstitial of cajal cell.

An acute increase twice or decrease (halve) in blood glucose can cause delayed or accelerated gastric emptying, respectively[14,18]. Additionally, the alterations in gastric emptying can produce fluctuations in glycemia, which then affects the gastric emptying rate, thus creating a vicious cycle. In the hyperglycemic state, pyloric contractions and antral hypomotility occur, leading to delayed emptying. On the other hand, hypoglycemia stimulates the vagus nerve[17].

Likewise, vagal dysfunction plays a role in diabetic gastroparesis. When food is ingested and gastric accommodation is disturbed, patients may experience symptoms such as early satiety, fullness, and discomfort. Vagal neuropathy can lead to reduced pyloric relaxation, impaired antral contraction, and impaired antropyloric coordination [19,20].

Alterations of the enteric nervous system also play an important role. The myenteric plexus contains a network of nerves found in layers between the longitudinal and circular intestinal muscular and coordinates gastric motor function. This myenteric plexus is made up of excitatory (cholinergic), inhibitory (nitrergic) motor neurons, primary afferent neurons, and interneurons[19]. Excitatory motor neurons induce muscle contractions by releasing neurotransmitters such as acetylcholine and substance P while, inhibitory neurons will relax muscle tissue by releasing nitric oxide, ATP, and vasoactive intestinal peptide[15]. Pathological changes in these pathways affect motor control and lead to delayed emptying, impaired accommodation, and gastric dysrhythmia[17].

Non-obese diabetic (NOD) mice showed a reversible loss of gastric nNOS expression, suggesting that in diabetic patients may exist negative regulation of nNOS without loss of nitrate neurons[15]. A study in rats administered streptozotocin (STZ) to induce diabetes found a reversible loss of nNOS after 4-8 wk, which progressed to irreversible loss due to apoptosis induced by oxidative stress after 12 wk. Because the

active nNOS enzyme is a dimerized protein, the loss of this dimerization can cause impaired neuromuscular function, as has been reported in the antrum of STZ-induced diabetic rats[21].

Loss of ICC has been reported in animal models and diabetic patients with gastroparesis. NOD mice and STZ-induced rats show a loss of ICC in both the body and the antrum[17]. The Gastroparesis Clinical Research Consortium in America collected data from patients with gastroparesis correlating cellular changes in surgical full-thickness gastric biopsies with the patient's symptoms[22]. A decrease in ICC and the gastric emptying rate was observed. In contrast to previous data in animals and humans, nNOS expression was not significantly decreased in diabetic patients. It should be noted that the full-thickness biopsies were taken from patients undergoing gastric neurostimulator placement, and therefore may represent a subgroup that is not representative of the general population with diabetic gastroparesis[22].

It is well known that diabetes induces a state of oxidative stress and contributes to the loss of nitrogen function. Increased oxidative stress in NOD mice due to loss of macrophage heme-oxygenase-1 (HO-1), which normally protects against free radicals in the enteric nervous system, was associated with loss of ICC inducing a delayed gastric emptying. The onset of delayed gastric emptying is associated with the loss of a subset of HO-1 macrophages. Induction of HO-1 reverses the delay in gastric emptying[2].

CLINICAL MANIFESTATIONS

The prevalence of gastrointestinal discomfort in diabetic patients is higher than in the general population, reaching up to 70% higher in some community studies[23].

Due to the alteration in the mechanisms of motility and secretion in the gastrointestinal tract, diabetic enteropathy could affect any portion of the gastrointestinal tract, including esophageal alterations, which are observed in more than 60% of diabetic patients[24,25].

Esophageal alterations

Esophageal motility disorders usually present with symptoms of gastroesophageal reflux or dysphagia (see Figure 4). However, it can also present as odynophagia, generally related to esophageal candidiasis[24].

In some cases, gastroesophageal reflux presents as a cough and worsening of respiratory parameters, delaying the diagnosis[25]. Heartburn is associated with gastroesophageal reflux in up to 41%[24].

Studies in patients with dysphagia showed that the symptoms are more frequent in women and white people. Also, about 45% of diabetic patients with dysphagia had esophageal motor abnormalities. When groups of patients with dysphagia were compared, diabetic patients had a higher percentage of smoking and body mass index than the group of non-diabetic patients with dysphagia. Manometry results indicated that patients with insulin treatment were associated with greater swallowing weakness, without significant association with glycosylated hemoglobin levels[25]. Acute hyperglycemic episodes are related to lower esophageal motility and greater dysphagia[12]. Besides, the presence of autonomic and peripheral neuropathy and retinopathy is associated with a higher frequency of erosive esophagitis compared to patients without neuropathy[26].

Gastric disorders

Clinical symptoms of gastroparesis include nausea, vomiting, early satiety, postprandial fullness, abdominal distension, belching, and upper abdominal discomfort. These symptoms may overlap with symptoms seen in functional dyspepsia[27,28].

The cardinal symptoms of gastroparesis usually present in combination, not individually. Although the symptoms of idiopathic and diabetic gastroparesis are similar, vomiting and early satiety are more frequent in diabetic gastroparesis; whereas that abdominal pain is more frequent in idiopathic gastroparesis[27].

A meta-analysis of 92 studies demonstrated that delayed gastric emptying is associated with gastrointestinal symptoms such as nausea (OR = 1.6, 95%CI: 1.4-1.8), vomiting (OR = 2.0, 95%CI: 1.6-2.7), early satiety (OR = 1.8, 95%CI: 1.2-2.6) and not significant with abdominal pain (OR = 1.5, 95%CI: 1.0-2.2) with an OR = 2.0; however, the abdominal pain had a lower association[29]. In patients with documented gastroparesis, the association between symptoms and delayed gastric emptying is less clear, finding nausea as a symptom. In diabetic patients, only a strong association was found

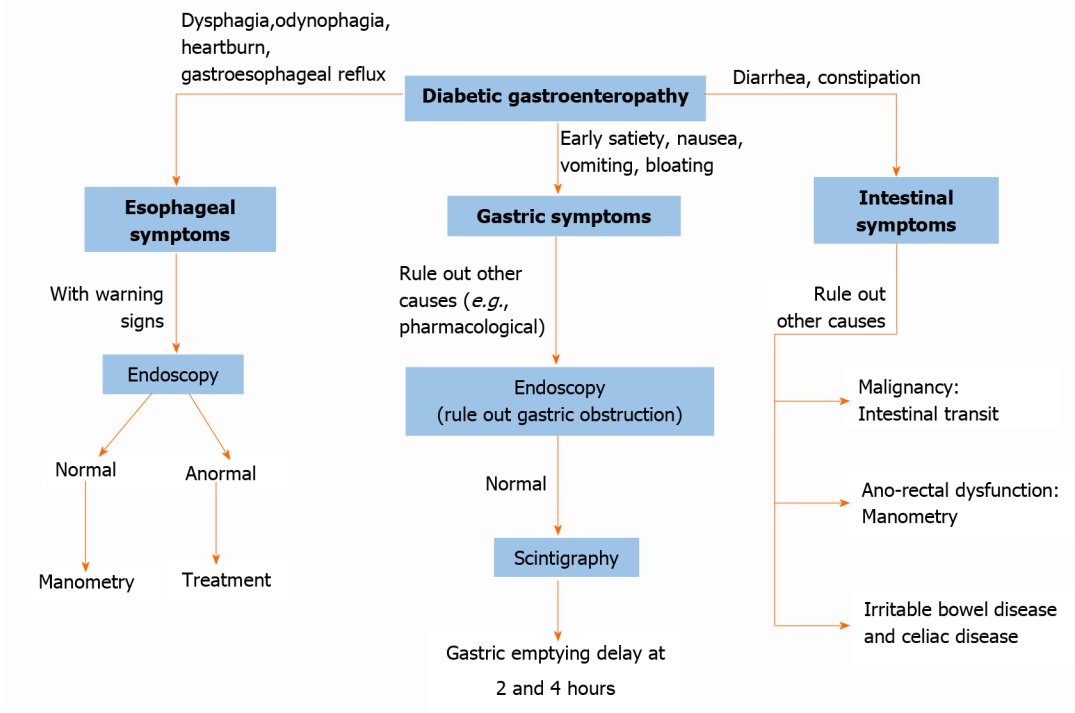

Figure 4 Diagnostic algorithm for diabetic gastroenteropathy.

between early satiety and delayed gastric emptying. Nevertheless, the neuropathy can result in gastric motility alterations and decreased symptoms.

In patients with type 1 diabetes have been observed that women are more likely to have gastroparesis than men (5.8% *vs* 3.5% $P < 0.001$), besides, patients with gastroparesis usually were older. Patients with gastroparesis generally had a longer duration of diabetes, higher Hemoglobin A1c levels, and more frequent episodes of severe hypoglycemia[30], the influence of gender is also observed in patients with type 2 diabetes. The reason for the difference in the prevalence of diabetic gastroparesis between men and women is unknown; however, gastric motility is dependent on the neuronal synthesis of nitric oxide, uncoupling of nitric oxide synthase causes a decreased synthesis of NO, leading to a reduction in smooth muscle relaxion. These events need a cofactor that is diminished in diabetic female rats, impairs NOS activity and this mechanism is probably influenced by estrogens[28].

Among the risk factors, obesity in diabetics is associated with an increase in the risk of approximately ten times. It has been reported that 50% of patients with idiopathic gastroparesis are overweight or obese, and the symptoms differ with body mass index. Obese patients have fewer symptoms of loss of appetite or inability to finish a meal but have higher rates of gastroesophageal reflux[12].

In 25-years follow-up of diabetic patients with gastrointestinal symptoms, it has been observed that both the symptoms and gastric emptying are relatively stable over time[23].

Intestinal disorders

The most common symptoms at the intestinal level are constipation, diarrhea, pain, and bloating. The frequency of chronic constipation is higher than chronic diarrhea in this group of patients (25% *vs* 5%)[27].

The NHANES study found that 25% of diabetics had gastrointestinal symptoms, but unlike previous studies, this one reported that only chronic diarrhea was more prevalent in diabetics than non-diabetics (11.2% *vs* 6.0%, $P < 0.0001$); However, the prevalence of chronic constipation there were no differences between diabetics and non-diabetics. Furthermore, diabetic patients with chronic diarrhea tended to use more hypoglycemic drugs, mainly metformin; and diabetics with chronic constipation had reduced kidney function. No significant relationship was found between intestinal symptoms and the presence of retinopathy, glycosylated hemoglobin levels, and duration of diabetes[31].

Diarrhea usually lasts more than 6 wk, watery, not associated with pain, not bloody, and its presentation related to the duration of diabetes is variable. It is more common

in women and develops about 8 years after the diagnosis of diabetes. It occurs with a frequency interval of normal stools or even constipation, with a sudden increase in volume and frequency. Nocturnal diarrhea and fecal incontinence are two of the most distinctive findings of diabetic diarrhea[3].

Rectum and anus disorders

Fecal incontinence occurs more frequently in diabetic patients it's related to the duration of diabetes and the presence of microvascular complications. In diabetic patients, internal anal sphincter tone and anal contraction pressures are reduced[27].

The prevalence of fecal incontinence varies in a range of 7%-15%; however, it is often not voluntarily manifested by patients. Intestinal disorders such as diarrhea are an independent risk factor for fecal incontinence. Besides, smoking, obesity, advanced age, sedentary lifestyle, and female sex are also risk factors for fecal incontinence[8].

SYMPTOMATIC EVALUATION SCALES

Due to the need to monitor gastrointestinal symptoms in patients, the use of questionnaires is preferred. These questionnaires have been changing in recent years; despite this, some studies related to treatment continue to use non-validated tools for gastrointestinal symptoms evaluation. Although the questionnaires are considered the standard for symptomatic evaluation, they are affected by recall bias, which is why these may not be optimal for monitoring changes in symptoms over time[28].

The questionnaires should specify the terms to be used, use an explicit language, evaluate all relevant symptoms, produce comparable results when evaluated in patients with stable symptoms, and detect clinically significant changes[32].

The most widely used questionnaires are the Gastrointestinal Symptom Severity Index (PAGI-SYM), Gastroparesis Cardinal Symptom Index (GCSI), Gastrointestinal Symptom Rating Scale (GSRS), Assessment of Constipation Symptom (PAC-SYM), and the Assessment of Constipation Quality of Life (PAC-QOL)[28].

PAGI-SYM assesses the severity of symptoms of the upper gastrointestinal tract. It contains 20 items and is divided into 6 sections: heartburn-regurgitation, postprandial fullness-early satiety, nausea-vomiting, abdominal distention, upper and lower abdominal pain. It's useful in the evaluation of gastroesophageal reflux, dyspepsia, and gastroparesis[24,28]. GCSI encompasses three scales that measure nausea-vomiting, postprandial fullness-early satiety, and abdominal distension. It's a 2-wk reminder, helps in assessing the severity of gastroparesis symptoms. To assess the response of gastroparesis to the treatment, the Gastroparesis Cardinal Symptom Index-Daily Diary (ANMS GCSI-DD) has been created[33].

GSRS contains 15 items combined into 5 groups: reflux, abdominal pain, indigestion, diarrhea, and constipation. It provides a broader perspective than the previous questionnaire; however, more research is still needed for an adequate correlation between the questionnaire scores and the objective measures of gastrointestinal disorders. Nevertheless, this instrument is not very sensitive for gastroesophageal reflux[23,33].

PAC-SYM and PAC-QoL were developed to assess the severity of symptoms and the quality of life in patients with constipation. The first is composed of 12 items and three groups: abdominal, rectal symptoms, and stool characteristics. The M-PAC-SYM questionnaire has been developed for patients with chronic constipation and could be more useful for the evaluation of functional constipation related to diabetes[34].

DIAGNOSIS

Esophageal disorders

The investigation of esophageal symptoms in diabetic patients is carried out in the same way as in non-diabetic patients. The typical symptoms of gastroesophageal reflux is sufficient for the diagnosis. Endoscopy and response to antisecretory therapy are not recommended as a diagnostic means. However, endoscopy shows its usefulness in the evaluation of complications in the mucosa, as well as in the detection of candidal esophagitis[23,31].

Esophageal motility disorders can be evaluated with manometry, and a video fluoroscopic swallowing exam. pH measurement studies can be useful in the evaluation of gastroesophageal reflux with or without impedance monitoring, which

allows the evaluation of air and liquid transit (see Figure 4)[23].

Manometry would show delays in esophageal transit times and reduced pressure in the lower esophageal sphincter. Manometry plus pH measurement serves for a better assess esophageal motility[23,25].

Gastric disorders

Gastric emptying scintigraphy is the method of choice to evaluate gastroparesis. In this method, the patient eats a food radiolabelled with Technetium-99, after which gastric emptying is measured. It can also be used to measure the transit time of the intestine and colon. Indications for gastric emptying scintigraphy include insulin-dependent diabetes and post-prandial symptoms or diabetes with poor glycemic control, non-ulcer-associated dyspepsia, severe esophagitis caused by reflux, nausea, vomiting, weight loss, upper abdominal discomfort, new early satiety, and evaluate treatment response with prokinetic drugs. After the ingestion of radiolabeled foods, liquids diffuse rapidly through the stomach, while solids are mainly concentrated in the fundus[35]. Normal results are gastric residual less than 60% at 2 h and less than 10% at 4 h. Higher values indicate gastroparesis. Gastric retention of more than 60% at 2 h has a sensitivity of 100% and specificity of 20%. Gastric retention of more than 10% at 4 h has a sensitivity of 100% and specificity of 70% for gastroparesis[36].

In the breath test, the non-radioactive isotope C13 is bound to a digestible substance, generally octanoic acid; which is mixed with solid food, absorbed in the proximal small intestine, with subsequent metabolization in the liver towards C13-C02, which is measurable in exhalation. The breath test has a sensitivity of 89% and a specificity of 80%. Compared to scintigraphy, the test is easier to perform and does not use radiation; however, concomitant diseases such as celiac disease affect profitability [28]. This test is carried out over a period of 4 h after 8-h fasting. Breath samples are collected before meals and collect breath samples every 30 min (see Figure 4)[37].

The wireless capsule continuously measures pressure, pH, and temperature as it moves through the gastrointestinal tract. The test involves a standardized meal and subsequent ingestion of the capsule. The data from the capsule is transmitted to a receiving unit. With this method, it has been observed that around 44% of type 1 diabetic patients with some degree of sensory-motor neuropathy had abnormal transit in one or more segments, independent of the presence of gastrointestinal symptoms [38]. The change in pH across the segments may represent fermentation in the cecal region that may influence colonic transit times. The test is limited to specialized centers and has high costs. It has only 52.5% concordant results with scintigraphy, so in patients with suspected gastroparesis additional investigations will be necessary[28, 37].

Other methods are the radiopaque markers (capsules containing plastic elements), which are ingested by the patient and followed through the gastrointestinal tract using plain abdominal radiographs. It is a widely available and useful test to detect significant variations in the time of intestinal transit and gastric emptying. Like the test mentioned above, a normal test does not exclude delayed gastric emptying, so additional tests are necessary[28].

The aforementioned tests should be performed when the presence of mechanical obstruction that could produce symptoms similar to gastroparesis has been ruled out [37].

Intestinal disorders

Due to one of the fundamental causes of gastrointestinal symptoms is an intestinal bacterial overgrowth, his diagnostic standard is the aspiration and culture of jejunal fluid. It requires the use of endoscopy and a high probability of external contamination and false-negative results. Breath tests may help, but they do not have adequate sensitivity[23,28,37].

In constipation, we can use anorectal manometry for the evaluation of defecation disorder. The measurement of intestinal transit using the tests described above may be justified too. A thorough investigation should be conducted to rule out malignancy (see Figure 4)[25].

Medication used by patients should be considered as a possible cause of diarrhea and constipation. Furthermore, due to the higher prevalence of the celiac disease in diabetic patients than in the general population, screening using serological tests is justified[23,25].

TREATMENT

There is currently no cure for diabetic gastroenteropathy. Therefore, the goals of treatment are to delay his progression of the disease, relieve symptoms, control complications, and restore function. The management of diabetic gastroenteropathy is multidisciplinary. It requires the participation of multiple specialists such as the gastroenterologist, endocrinologist, nutritionist, psychologist, interventional radiologist, and surgeon[23].

In a didactic way, we have divided the management of gastroparesis and diabetic enteropathy, taking into account that both forms of the disease can coexist.

MANAGEMENT OF DIABETIC GASTROPARESIS

Most patients tend to have a mild-moderate disease and therefore respond to nutritional recommendations, dietary modifications, and adequate glycemic control, as well as prokinetic agents or antiemetic medications (with variable efficacy)[39] if there is no response to the initial measurements[3,40]. A small percentage of patients have severe disease characterized by inadequate oral tolerance, chronic malnutrition, weight loss, and frequent hospitalizations. Representing management as a therapeutic challenge[3].

On the other hand, it is also important to manage the comorbidities that usually occur in patients with diabetic gastropareses, such as gastroesophageal reflux, intestinal dysmotility, deficiency of vitamin D, and other micronutrients, bacterial and fungal infections of the gastrointestinal tract, and macrovascular-microvascular complications of Diabetes Mellitus[41,42].

Nutritional recommendations

Dietary modifications constitute the first-line management for gastroparesis, such as a decrease in particle size diet reduces upper gastrointestinal symptoms in patients with diabetic gastroparesis, even though their efficacy has not been clearly established[43,44].

Frequently, patients with diabetic gastroparesis tend to have a lower caloric intake than recommended, as well as significant micronutrient and macronutrient deficiencies[45]. A diet based on a low content of simple sugars and foods rich in fiber, as proposed by the American Diabetes Association (ADA) for diabetic patients[46], is not usually useful for patients with diabetic gastroparesis[3].

Measures to promote gastric emptying or do not delay it is recommended[3]. That is why the consumption of fats, fibers, and carbonated liquids, which should delay gastric emptying, should be minimized[37]. A recommended and staggered approach is to start with liquids with high nutritional value, followed by soups and shakes, and later to introduce solid foods that do not delay gastric emptying[3]. Low-fat foods should be encouraged 4-5 times per day. Patients should be instructed to drink fluids with meals and sit or walk for 1-2 h after meals.

If all these nutritional recommendations are ineffective, the patient should be suggested to consume the total calories in liquids, soups, or shakes, since fluid emptying is frequently preserved[42].

It is necessary to emphasize that all these dietary changes require education to the patients and their families. It has been found that the prevalence of nutritional consultations in diabetic patients with gastroparesis is less than 40%[45].

Glycemic control

It is vitally important to optimize glycemic control to minimize the acute symptoms of diabetic gastroparesis and to improve gastric emptying[3,44]. Random serum to avoid inhibition of gastric myoelectric motility and control[3].

The treatment used to achieve adequate glycemic control must be individualized[3,44]. Oral antidiabetics are not recommended for patients with type 2 diabetes with clinically significant diabetic gastroparesis. The pharmacokinetics of these drugs are affected by delayed gastric emptying; therefore, agents are not ideal for effective glycemic control[3,44].

Likewise, the adverse effects of antidiabetic medications also play an important role, such as gastrointestinal intolerance frequently reported with metformin, hypoglycemia caused by sulfonylureas, diarrhea and abdominal distension due to alpha-glucosidase inhibitors, inconsistent effect inhibitors of dipeptidyl peptidase-4 on gastric emptying, and an unclear impact of SGLT-2 inhibitors. In relation to injectable therapy, GLP-1

agonists can exacerbate symptoms of delayed gastric emptying[3,44].

Patients with diabetic gastroparesis by type 1 diabetes and most of the patients with type 2 diabetes will require insulin for glycemic control[47]. It is recommended to administer prandial insulin after meals to prevent postprandial hypoglycemia if food is not fully consumed. Multiple intakes of small foods, aggressive glucose monitoring, and frequent small doses of rapid-acting insulins are recommended to prevent postprandial hyperglycemia[3]. Currently, the recommended treatment for glycemic control in patients with diabetic gastroparesis who receive insulin is continuous subcutaneous insulin infusion, based on optimizing glycemic control and reducing hospitalizations[48]; however, we must not forget the economic cost that this usually entails. The use of premixed insulins is not recommended in this group of patients[9].

Pharmacologic treatment

The drugs most used in the treatment of diabetic gastroparesis usually include prokinetics and antiemetics[3]. Several novel targeted therapies are still under investigation (see Figure 5).

Prokinetics: Metoclopramide is a D-2 receptor antagonist with antiemetic and prokinetic effects that increase antral contractions and coordinate antral and duodenal motility. The maximum daily dose is 40 mg/d. It can be used parenterally when symptoms are severe. Among the adverse effects is an increase in serum prolactin concentration. Gynecomastia and galactorrhea can occur in adults, adolescents, and children; while, adult women can develop oligomenorrhea. Likewise, it can stimulate aldosterone synthesis and cause uncontrolled hypertension in patients with primary hyperaldosteronism, and it can also prolong the QT interval in susceptible patients[3]. It is the only drug approved by the United States Food and Drug Administration (FDA) for the management of gastroparesis; however, in February 2009, the FDA and the European Medicines Agency established black box warnings for long-term use (more than 12 wk) of metoclopramide due to the risk of irreversible tardive dyskinesia, which has limited its use[40]. Domperidone is another dopamine 2 receptor antagonist, identical to metoclopramide, with equal efficacy to the latter, but with fewer adverse effects on the central nervous system; because it does not cross the blood-brain barrier[49]. Daily doses between 10-30 mg, administered 30 min before meals and at bedtime, have been found to reduce gastrointestinal upset and hospitalizations due to gastroparesis.

Erythromycin is a macrolide with an agonist effect on motilin receptors in the gastrointestinal tract. It increases gastric emptying in a dose-dependent manner. It has been shown to stimulate gastric emptying in patients with diabetic gastroparesis. Daily dose 50-100 mg administered 3 times a day, in combination with low-volume diets can be effective for controlling gastroparesis. Cases of QT interval prolongation have also been found[11,3].

Cisapride is a potent prokinetic agent that, acting in the stomach through 5-hydroxytryptamine receptors, accelerates gastric emptying from solid foods and improves dyspeptic symptoms[3].

There are future prokinetic drugs in development, among which motilin agonists, ghrelin agonists, and new type 4 5-hydroxytryptamine receptor agonists[11,3].

Antiemetics: Both nausea and vomiting are often the most disabling symptoms in patients with diabetic gastroparesis. Antiemetic drugs can be serotonin antagonists such as ondansetron, at doses of 4-8 mg twice a day, or type 1 histamine receptor antagonists, such as dimenhydrinate at a dose of 50 mg 4 times a day. Both classes of drugs are often used alone or in combination with prokinetic agents[3].

Other treatment modalities

Botulinum toxin, a potent inhibitor of neuromuscular transmission, has been postulated to improve gastric emptying and symptoms for several months; however, double-blind randomized studies have shown that improves gastric emptying, it does not improve symptoms[50].

Likewise, there has been an interest in the pylorus role in delayed gastric emptying, which is why some authors propose gastric peroral endoscopic myotomy (G-POEM) as a treatment modality in refractory patients, be it surgical or endoscopic. P. Mekaroonkamol reported 03 cases of gastroparesis refractory to conventional treatment, of varied etiology (*e.g.*, postinfectious, postsurgical, or idiopathic) that progressed favorably after undergoing G-POEM as salvage therapy[51].

For the select group of patients with severe and refractory disease, gastric electrical stimulation may be an option. It has been shown to improve nausea, vomiting, quality

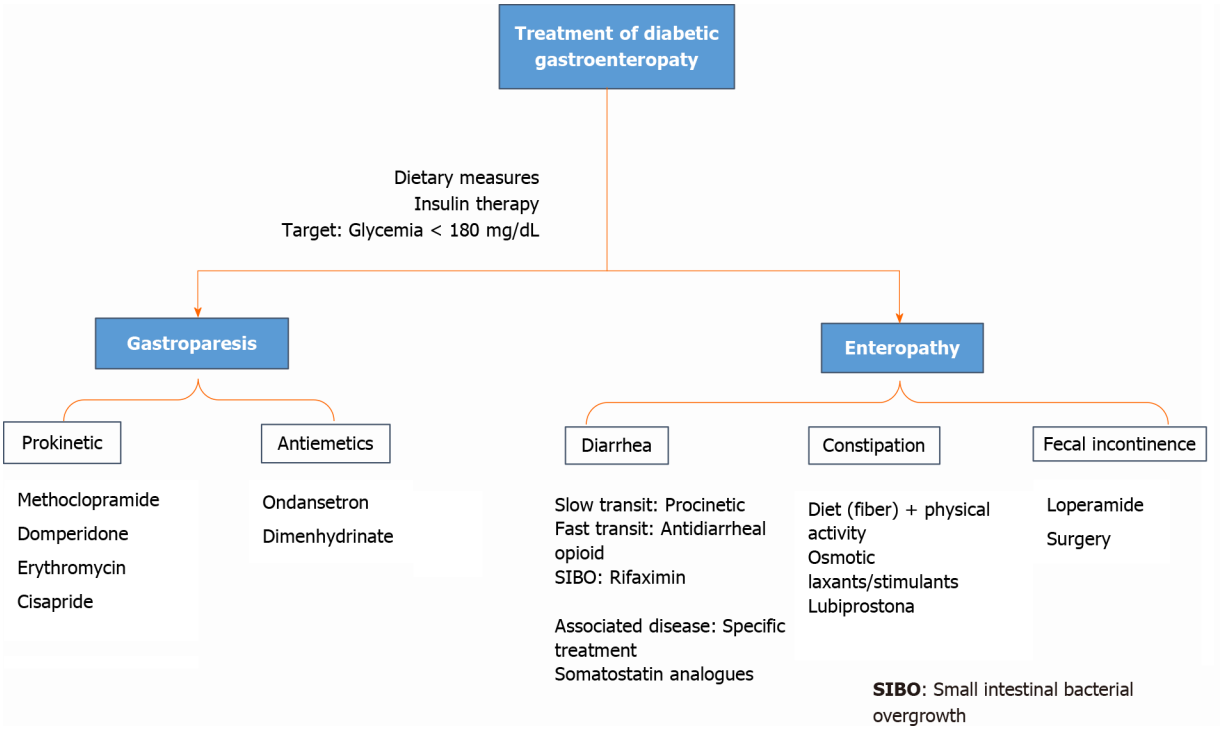

Figure 5 Management of diabetic gastroenteropathy.

of life, and nutritional status after sixth month of treatment[52,53].

A significant number of patients fail medical management and require surgical treatment. The main role of surgery is the relief of symptoms, decompression of the stomach, access to enteral nutrition, and stimulating gastric emptying[3].

MANAGEMENT OF DIABETIC ENTEROPATHY

The alteration of the intestine by diabetes mellitus has a variety of presentations: chronic diarrhea (involvement of the small intestine), constipation when it affects the colon, and also fecal incontinence (see Figure 4). All of them are explained by the multisystemic nature of the disease (autonomic neuropathy, infectious involvement, and autoimmune disease when in the presence of type 1 diabetes)[40,51,54].

Chronic diarrhea

The prevalence of chronic diarrhea in diabetic patients ranges from 3.7% to 22%[54]; compared to the general population, they have around twice the risk of having diarrhea (11% *vs* 6%)[33]. An important part of the management is to assess the hydration status and electrolyte imbalance that management would need. As in gastroparesis, the goal is to achieve good glycemic control and diet management. If these initial measures fail, drug therapy with opioid group antidiarrheals is an option they can be administered with caution due to their toxicity: megacolon and the potential worsening of bacterial overgrowth[12].

For small intestinal bacterial overgrowth, antibiotic therapy should be started. Rifaximin is the best research-based agent for this disorder works selectively on the gastrointestinal tract, lowers resistance, and improves symptoms in 33% to 99% of patients[55]. Somatostatin analogs like octreotide and lanreotide also improve symptoms[10]. Another common cause of diarrhea in diabetic patients is the use of drugs such as metformin, which reduces the ileal absorption of bile salts, artificial sweeteners through an osmotic mechanism (seen mainly with sorbitol intake greater than 20 g), it is split by the intestinal flora that produces hydrogen and short-chain fatty acids, which are the cause of diarrhea associated with this substance[56].

Nevertheless, there is no specific treatment for chronic diarrhea in diabetic patients. If a slow transit is targeted, the option would be prokinetics, while if the symptoms are in favor of rapid transit, the choice would be opiates. The use of somatostatin analogs that inhibit water secretion, increase absorptive capacity, and suppress the release of

hormones with gastrointestinal action; is indicated in the failure of conventional therapies[56].

Constipation

When constipation is the main complaint, good hydration, high-fiber meals, and routine physical activities are the first recommendations to make. Randomized placebo-controlled clinical trials are showing that the intake of natural psyllium (10 g twice a day) or flaxseed (10 g twice a day) reduces constipation symptoms and improves glycemic control in people with diabetes type 2. There are no studies specifically investigating the effects of laxatives in people with constipation as a complication due to diabetic gastroenteropathy[57,58]. Treatment focuses on its symptomatic management, with the use of a diet that promotes softening of the stool, and the use of laxatives that increase intestinal transit.

Although without much solid evidence, we can suggest starting using osmotic laxatives such as polyethylene glycol; if insufficient, stimulant laxatives such as bisacodyl or picosulfate can be added. Lubiprostone, a chloride channel activator, increases secretion from the colon, reducing colonic transit time and increasing the number of spontaneous bowel movements in people with diabetes-related constipation[48,59].

Fecal incontinence

In the management of fecal incontinence, which is often aggravated by diarrhea, a priority is identifying an underlying cause of diarrhea and addressing it. Often improves on its own within good glycemic control[10]. Otherwise, a dietary intervention might be recommended, and as it is largely associated with neuropathy and reduced sensitivity of the anal canal, loperamide, suppositories, or enemas should be considered. Treatment of refractory cases is very complex and may even require a stoma[48,51].

Neuromodulatory electrical stimulation of the sacral nerve is an emerging technique of treatment for fecal incontinence and possible sensitivity in the anal canal. However, it has not been specifically investigated in people with diabetic gastroenteropathy[60].

CONCLUSION

Diabetes mellitus is a chronic disease with a rising prevalence worldwide, as are its complications, including gastroenteropathy. Its pathophysiology integrates hyperglycemia, vagal dysfunction, loss of expression of neural nitric oxide synthase in the myenteric plexus, alterations in the interstitial cell network of Cajal, and oxidative stress. The clinical features are gastroesophageal reflux, gastroparesis, constipation, abdominal pain, and diarrhea. Among the diagnostic studies, manometry together with pH measurement (evaluating esophageal motility), gastric emptying scintigraphy, breath test (evaluating gastroparesis), aspiration, and jejunal culture (evaluating bacterial overgrowth) stand out. There is no definitive treatment for diabetic gastroenteropathy- The multidisciplinary approach is considered to seek to slow the progression of the disease, alleviate symptoms, and restore function. A diet low in simple sugars and high in fiber is recommended; optimize glycemic control, with a target glycemia of less than 180 mg/dL. Regarding drug therapy, prokinetics and antiemetics are included, and if bacterial overgrowth occurs, antibiotic treatment with Rifaximin. Current evidence is accumulating among the new approaches, including the use of botulinum toxin, pyroloplasty, and electrical gastric stimulation in selected patients. Although there are new techniques for diagnosis, but it does not appear a definitive cure in the near future, remaining a concern, well designed clinical trials are needed in this field.

ACKNOWLEDGEMENTS

The research team appreciates the contributions, suggestions, and corrections made by Dr. Julio Hilario Vargas, to publish this manuscript.

REFERENCES

1 **Kumar M**, Chapman A, Javed S, Alam U, Malik RA, Azmi S. The Investigation and Treatment of Diabetic Gastroparesis. *Clin Ther* 2018; **40**: 850-861 [PMID: 29748143 DOI: 10.1016/j.clinthera.2018.04.012]

2 **Borgoño CA**, Zinman B. Insulins: past, present, and future. *Endocrinol Metab Clin North Am* 2012; **41**: 1-24 [PMID: 22575404 DOI: 10.1016/j.ecl.2012.03.002]

3 **Quianzon CC**, Cheikh I. History of insulin. *J Community Hosp Intern Med Perspect* 2012; **2** [PMID: 23882369 DOI: 10.3402/jchimp.v2i2.18701]

4 **Shen S**, Xu J, Lamm V, Vachaparambil CT, Chen H, Cai Q. Diabetic Gastroparesis and Nondiabetic Gastroparesis. *Gastrointest Endosc Clin N Am* 2019; **29**: 15-25 [PMID: 30396524 DOI: 10.1016/j.giec.2018.08.002]

5 **Krishnasamy S**, Abell TL. Diabetic Gastroparesis: Principles and Current Trends in Management. *Diabetes Ther* 2018; **9**: 1-42 [PMID: 29934758 DOI: 10.1007/s13300-018-0454-9]

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

7 **Jung HK**, Choung RS, Locke GR 3rd, Schleck CD, Zinsmeister AR, Szarka LA, Mullan B, Talley NJ. The incidence, prevalence, and outcomes of patients with gastroparesis in Olmsted County, Minnesota, from 1996 to 2006. *Gastroenterology* 2009; **136**: 1225-1233 [PMID: 19249393 DOI: 10.1053/j.gastro.2008.12.047]

8 **Choung RS**, Locke GR 3rd, Schleck CD, Zinsmeister AR, Melton LJ 3rd, Talley NJ. Risk of gastroparesis in subjects with type 1 and 2 diabetes in the general population. *Am J Gastroenterol* 2012; **107**: 82-88 [PMID: 22085818 DOI: 10.1038/ajg.2011.310]

9 **Koch KL**, Calles-Escandón J. Diabetic gastroparesis. *Gastroenterol Clin North Am* 2015; **44**: 39-57 [PMID: 25667022 DOI: 10.1016/j.gtc.2014.11.005]

10 **Kurniawan AH**, Suwandi BH, Kholili U. Diabetic Gastroenteropathy: A Complication of Diabetes Mellitus. *Acta Med Indones* 2019; **51**: 263-271 [PMID: 31699951]

11 **Goyal RK**, Guo Y, Mashimo H. Advances in the physiology of gastric emptying. *Neurogastroenterol Motil* 2019; **31**: e13546 [PMID: 30740834 DOI: 10.1111/nmo.13546]

12 **Sullivan A**, Temperley L, Ruban A. Pathophysiology, Aetiology and Treatment of Gastroparesis. *Dig Dis Sci* 2020; **65**: 1615-1631 [PMID: 32350720 DOI: 10.1007/s10620-020-06287-2]

13 **Kuo P**, Rayner CK, Jones KL, Horowitz M. Pathophysiology and management of diabetic gastropathy: a guide for endocrinologists. *Drugs* 2007; **67**: 1671-1687 [PMID: 17683169 DOI: 10.2165/00003495-200767120-00003]

14 **Schvarcz E**, Palmér M, Aman J, Horowitz M, Stridsberg M, Berne C. Physiological hyperglycemia slows gastric emptying in normal subjects and patients with insulin-dependent diabetes mellitus. *Gastroenterology* 1997; **113**: 60-66 [PMID: 9207262 DOI: 10.1016/s0016-5085(97)70080-5]

15 **Watkins CC**, Sawa A, Jaffrey S, Blackshaw S, Barrow RK, Snyder SH, Ferris CD. Insulin restores neuronal nitric oxide synthase expression and function that is lost in diabetic gastropathy. *J Clin Invest* 2000; **106**: 373-384 [PMID: 10930440 DOI: 10.1172/JCI8273]

16 **Stevens JE**, Jones KL, Rayner CK, Horowitz M. Pathophysiology and pharmacotherapy of gastroparesis: current and future perspectives. *Expert Opin Pharmacother* 2013; **14**: 1171-1186 [PMID: 23663133 DOI: 10.1517/14656566.2013.795948]

17 **Vanormelingen C**, Tack J, Andrews CN. Diabetic gastroparesis. *Br Med Bull* 2013; **105**: 213-230 [PMID: 23363458 DOI: 10.1093/bmb/Ldt003]

18 **Fraser RJ**, Horowitz M, Maddox AF, Harding PE, Chatterton BE, Dent J. Hyperglycaemia slows gastric emptying in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1990; **33**: 675-680 [PMID: 2076799 DOI: 10.1007/BF00400569]

19 **Meldgaard T**, Keller J, Olesen AE, Olesen SS, Krogh K, Borre M, Farmer A, Brock B, Brock C, Drewes AM. Pathophysiology and management of diabetic gastroenteropathy. *Therap Adv Gastroenterol* 2019; **12**: 1756284819852047 [PMID: 31244895 DOI: 10.1177/1756284819852047]

20 **Nguyen LA**, Snape WJ Jr. Clinical presentation and pathophysiology of gastroparesis. *Gastroenterol Clin North Am* 2015; **44**: 21-30 [PMID: 25667020 DOI: 10.1016/j.gtc.2014.11.003]

21 **Gangula PR**, Maner WL, Micci MA, Garfield RE, Pasricha PJ. Diabetes induces sex-dependent changes in neuronal nitric oxide synthase dimerization and function in the rat gastric antrum. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G725-G733 [PMID: 17347455 DOI: 10.1152/ajpgi.00406.2006]

22 **Grover M**, Bernard CE, Pasricha PJ, Lurken MS, Faussone-Pellegrini MS, Smyrk TC, Parkman HP, Abell TL, Snape WJ, Hasler WL, McCallum RW, Nguyen L, Koch KL, Calles J, Lee L, Tonascia J, Ünalp-Arida A, Hamilton FA, Farrugia G; NIDDK Gastroparesis Clinical Research Consortium (GpCRC). Clinical-histological associations in gastroparesis: results from the Gastroparesis Clinical Research Consortium. *Neurogastroenterol Motil* 2012; **24**: 531-539, e249 [PMID: 22339929 DOI: 10.1111/j.1365-2982.2012.01894.x]

23 **Du YT**, Rayner CK, Jones KL, Talley NJ, Horowitz M. Gastrointestinal Symptoms in Diabetes: Prevalence, Assessment, Pathogenesis, and Management. *Diabetes Care* 2018; **41**: 627-637 [PMID: 29463666 DOI: 10.2337/dc17-1536]

24 **Chedid V**, Brandler J, Vijayvargiya P, Park SY, Szarka LA, Camilleri M. Characterization of Upper Gastrointestinal Symptoms, Gastric Motor Functions, and Associations in Patients with Diabetes at a Referral Center. *Am J Gastroenterol* 2019; **114**: 143-154 [PMID: 30166634 DOI: 10.1038/s41395-018-0234-1]

25 **Iyer PG**, Borah BJ, Heien HC, Das A, Cooper GS, Chak A. Association of Barrett's esophagus with type II Diabetes Mellitus: results from a large population-based case-control study. *Clin Gastroenterol Hepatol* 2013; **11**: 1108-1114. e5 [PMID: 23591277 DOI: 10.1016/j.cgh.2013.03.024]

26 **George NS**, Rangan V, Geng Z, Khan F, Kichler A, Gabbard S, Ganocy S, Fass R. Distribution of Esophageal Motor Disorders in Diabetic Patients With Dysphagia. *J Clin Gastroenterol* 2017; **51**: 890-895 [PMID: 28746079 DOI: 10.1097/MCG.0000000000000894]

27 **Marathe CS**, Rayner CK, Wu T, Jones KL, Horowitz M, Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, Dungan K, Grossman A, Hershman JM, Hofland J, Kalra S, Kaltsas G, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, McGee EA, McLachlan R, Morley JE, New M, Purnell J, Sahay R, Singer F, Stratakis CA, Trence DL, Wilson DP. Gastrointestinal Disorders in Diabetes 2000 [PMID: 31986000]

28 **Meldgaard T**, Olesen SS, Farmer AD, Krogh K, Wendel AA, Brock B, Drewes AM, Brock C. Diabetic Enteropathy: From Molecule to Mechanism-Based Treatment. *J Diabetes Res* 2018; **2018**: 3827301 [PMID: 30306092 DOI: 10.1155/2018/3827301]

29 **Vijayvargiya P**, Jameie-Oskooei S, Camilleri M, Chedid V, Erwin PJ, Murad MH. Association between delayed gastric emptying and upper gastrointestinal symptoms: a systematic review and meta-analysis. *Gut* 2019; **68**: 804-813 [PMID: 29860241 DOI: 10.1136/gutjnl-2018-316405]

30 **Aleppo G**, Calhoun P, Foster NC, Maahs DM, Shah VN, Miller KM; T1D Exchange Clinic Network. Reported gastroparesis in adults with type 1 diabetes (T1D) from the T1D Exchange clinic registry. *J Diabetes Complications* 2017; **31**: 1669-1673 [PMID: 28989086 DOI: 10.1016/j.jdiacomp.2017.08.014]

31 **Sommers T**, Mitsuhashi S, Singh P, Hirsch W, Katon J, Ballou S, Rangan V, Cheng V, Friedlander D, Iturrino J, Lembo A, Nee J. Prevalence of Chronic Constipation and Chronic Diarrhea in Diabetic Individuals in the United States. *Am J Gastroenterol* 2019; **114**: 135-142 [PMID: 30410038 DOI: 10.1038/s41395-018-0418-8]

32 **Mouli VP**, Ahuja V. Questionnaire based gastroesophageal reflux disease (GERD) assessment scales. *Indian J Gastroenterol* 2011; **30**: 108-117 [PMID: 21785994 DOI: 10.1007/s12664-011-0105-9]

33 **Revicki DA**, Camilleri M, Kuo B, Szarka LA, McCormack J, Parkman HP. Evaluating symptom outcomes in gastroparesis clinical trials: validity and responsiveness of the Gastroparesis Cardinal Symptom Index-Daily Diary (GCSI-DD). *Neurogastroenterol Motil* 2012; **24**: 456-463, e215 [PMID: 22284754 DOI: 10.1111/j.1365-2982.2012.01879.x]

34 **Neri L**, Conway PM, Basilisco G; Laxative Inadequate Relief Survey (LIRS) Group. Confirmatory factor analysis of the Patient Assessment of Constipation-Symptoms (PAC-SYM) among patients with chronic constipation. *Qual Life Res* 2015; **24**: 1597-1605 [PMID: 25522976 DOI: 10.1007/s11136-014-0886-2]

35 **Alipour Z**, Khatib F, Tabib SM, Javadi H, Jafari E, Aghaghazvini L, Mahmoud-Pashazadeh A, Nabipour I, Assadi M. Assessment of the Prevalence of Diabetic Gastroparesis and Validation of Gastric Emptying Scintigraphy for Diagnosis. *Mol Imaging Radionucl Ther* 2017; **26**: 17-23 [PMID: 28291006 DOI: 10.4274/mirt.61587]

36 **Farrell MB**. Gastric Emptying Scintigraphy. *J Nucl Med Technol* 2019; **47**: 111-119 [PMID: 31167827 DOI: 10.2967/jnmt.117.227892]

37 **Camilleri M**. Clinical practice. Diabetic gastroparesis. *N Engl J Med* 2007; **356**: 820-829 [PMID: 17314341 DOI: 10.1056/NEJMcp062614]

38 **Farmer AD**, Pedersen AG, Brock B, Jakobsen PE, Karmisholt J, Mohammed SD, Scott SM, Drewes AM, Brock C. Type 1 diabetic patients with peripheral neuropathy have pan-enteric prolongation of gastrointestinal transit times and an altered caecal pH profile. *Diabetologia* 2017; **60**: 709-718 [PMID: 28105520 DOI: 10.1007/s00125-016-4199-6]

39 **Asha MZ**, Khalil SFH. Pharmacological Approaches to Diabetic Gastroparesis: A systematic review of randomised clinical trials. *Sultan Qaboos Univ Med J* 2019; **19**: e291-e304 [PMID: 31897312 DOI: 10.18295/SQUMJ.2019.19.04.004]

40 **Careyva B**, Stello B. Diabetes Mellitus: Management of Gastrointestinal Complications. *Am Fam Physician* 2016; **94**: 980-986 [PMID: 28075092]

41 **Kedar A**, Nikitina Y, Henry OR, Abell KB, Vedanarayanan V, Griswold ME, Subramony C, Abell TL. Gastric dysmotility and low serum vitamin D levels in patients with gastroparesis. *Horm Metab Res* 2013; **45**: 47-53 [PMID: 22956309 DOI: 10.1055/s-0032-1323689]

42 **Ajumobi AB**, Griffin RA. Diabetic gastroparesis: evaluation and management. *Hosp Physician* 2008; **44**: 27-35 [DOI: 10.12968/hmed.2012.73.8.451]

43 **Olausson EA**, Störsrud S, Grundin H, Isaksson M, Attvall S, Simrén M. A small particle size diet reduces upper gastrointestinal symptoms in patients with diabetic gastroparesis: a randomized controlled trial. *Am J Gastroenterol* 2014; **109**: 375-385 [PMID: 24419482 DOI: 10.1038/ajg.2013.453]

44 **Jalleh R**, Marathe CS, Rayner CK, Jones KL, Horowitz M. Diabetic Gastroparesis and Glycaemic Control. *Curr Diab Rep* 2019; **19**: 153 [PMID: 31792708 DOI: 10.1007/s11892-019-1281-8]

45 **Parkman HP**, Yates KP, Hasler WL, Nguyan L, Pasricha PJ, Snape WJ, Farrugia G, Calles J, Koch KL, Abell TL, McCallum RW, Petito D, Parrish CR, Duffy F, Lee L, Unalp-Arida A, Tonascia J,

Hamilton F; NIDDK Gastroparesis Clinical Research Consortium. Dietary intake and nutritional deficiencies in patients with diabetic or idiopathic gastroparesis. *Gastroenterology* 2011; **141**: 486-498, 498.e1-498. e7 [PMID: 21684286 DOI: 10.1053/j.gastro.2011.04.045]

46 **American Diabetes Association**. 3. Prevention or Delay of Type 2 Diabetes: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020; **43**: S32-S36 [PMID: 31862746 DOI: 10.2337/dc20-S003]

47 **Bennett WL**, Maruthur NM, Singh S, Segal JB, Wilson LM, Chatterjee R, Marinopoulos SS, Puhan MA, Ranasinghe P, Block L, Nicholson WK, Hutfless S, Bass EB, Bolen S. Comparative effectiveness and safety of medications for type 2 diabetes: an update including new drugs and 2-drug combinations. *Ann Intern Med* 2011; **154**: 602-613 [PMID: 21403054 DOI: 10.7326/0003-4819-154-9-201105030-00336]

48 **Sharma D**, Morrison G, Joseph F, Purewal TS, Weston PJ. The role of continuous subcutaneous insulin infusion therapy in patients with diabetic gastroparesis. *Diabetologia* 2011; **54**: 2768-2770 [PMID: 21842427 DOI: 10.1007/s00125-011-2282-6]

49 **Patterson D**, Abell T, Rothstein R, Koch K, Barnett J. A double-blind multicenter comparison of domperidone and metoclopramide in the treatment of diabetic patients with symptoms of gastroparesis. *Am J Gastroenterol* 1999; **94**: 1230-1234 [PMID: 10235199 DOI: 10.1111/j.1572-0241.1999.00456.x]

50 **Lacy BE**, Crowell MD, Schettler-Duncan A, Mathis C, Pasricha PJ. The treatment of diabetic gastroparesis with botulinum toxin injection of the pylorus. *Diabetes Care* 2004; **27**: 2341-2347 [PMID: 15451898 DOI: 10.2337/diacare.27.10.2341]

51 **Mekaroonkamol P**, Li LY, Dacha S, Xu Y, Keilin SD, Willingham FF, Cai Q. Gastric peroral endoscopic pyloromyotomy (G-POEM) as a salvage therapy for refractory gastroparesis: a case series of different subtypes. *Neurogastroenterol Motil* 2016; **28**: 1272-1277 [PMID: 27197717 DOI: 10.1111/nmo.12854]

52 **Abell T**, McCallum R, Hocking M, Koch K, Abrahamsson H, Leblanc I, Lindberg G, Konturek J, Nowak T, Quigley EM, Tougas G, Starkebaum W. Gastric electrical stimulation for medically refractory gastroparesis. *Gastroenterology* 2003; **125**: 421-428 [PMID: 12891544 DOI: 10.1016/s0016-5085(03)00878-3]

53 **Abell TL**, Johnson WD, Kedar A, Runnels JM, Thompson J, Weeks ES, Minocha A, Griswold ME. A double-masked, randomized, placebo-controlled trial of temporary endoscopic mucosal gastric electrical stimulation for gastroparesis. *Gastrointest Endosc* 2011; **74**: 496-503. e3 [PMID: 21872708 DOI: 10.1016/j.gie.2011.05.022]

54 **Lysy J**, Israeli E, Goldin E. The prevalence of chronic diarrhea among diabetic patients. *Am J Gastroenterol* 1999; **94**: 2165-2170 [PMID: 10445544 DOI: 10.1111/j.1572-0241.1999.01289.x]

55 **Pimentel M**. Review of rifaximin as treatment for SIBO and IBS. *Expert Opin Investig Drugs* 2009; **18**: 349-358 [PMID: 19243285 DOI: 10.1517/13543780902780175]

56 **Ebert EC**. Gastrointestinal complications of diabetes mellitus. *Dis Mon* 2005; **51**: 620-663 [PMID: 16458726 DOI: 10.1016/j.disamonth.2005.11.002]

57 **Noureddin S**, Mohsen J, Payman A. Effects of psyllium vs. placebo on constipation, weight, glycemia, and lipids: A randomized trial in patients with type 2 diabetes and chronic constipation. *Complement Ther Med* 2018; **40**: 1-7 [PMID: 30219432 DOI: 10.1016/j.ctim.2018.07.004]

58 **Soltanian N**, Janghorbani M. A randomized trial of the effects of flaxseed to manage constipation, weight, glycemia, and lipids in constipated patients with type 2 diabetes. *Nutr Metab (Lond)* 2018; **15**: 36 [PMID: 29760761 DOI: 10.1186/s12986-018-0273-z]

59 **Christie J**, Shroff S, Shahnavaz N, Carter LA, Harrison MS, Dietz-Lindo KA, Hanfelt J, Srinivasan S. A Randomized, Double-Blind, Placebo-Controlled Trial to Examine the Effectiveness of Lubiprostone on Constipation Symptoms and Colon Transit Time in Diabetic Patients. *Am J Gastroenterol* 2017; **112**: 356-364 [PMID: 27922028 DOI: 10.1038/ajg.2016.531]

60 **Haas S**, Brock C, Krogh K, Gram M, Lundby L, Drewes AM, Laurberg S. Does Sacral Nerve Stimulation Improve Continence Through Enhanced Sensitivity of the Anal Canal? *Dis Colon Rectum* 2016; **59**: 1039-1046 [PMID: 27749479 DOI: 10.1097/DCR.0000000000000677]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 810-826

DOI: 10.4239/wjd.v12.i6.810

ISSN 1948-9358 (online)

MINIREVIEWS

Endothelial impairment evaluation by peripheral arterial tonometry in pediatric endocrinopathies: A narrative review

Alberto La Valle, Marco Crocco, Decimo Silvio Chiarenza, Mohamad Maghnie, Giuseppe d'Annunzio

ORCID number: Alberto La Valle 0000-0003-3447-9163; Marco Crocco 0000-0001-7277-4767; Decimo Silvio Chiarenza 0000-0002-6877-9511; Mohamad Maghnie 0000-0002-7183-5238; Giuseppe d'Annunzio 0000-0002-1856-2277.

Author contributions: La Valle A, Crocco M, Chiarenza DS, d'Annunzio G searched the literature and drafted the manuscript; Maghnie M and d'Annunzio G revised the manuscript, provided further comments and editing.

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

Manuscript source: Invited

Alberto La Valle, Marco Crocco, Decimo Silvio Chiarenza, Mohamad Maghnie, Giuseppe d'Annunzio, Pediatric Clinic and Endocrinology, IRCCS Istituto Giannina Gaslini, Genoa16147, Italy

Alberto La Valle, Marco Crocco, Decimo Silvio Chiarenza, Mohamad Maghnie, Pediatric Clinic and Endocrinology, IRCCS Giannina Gaslini Institute, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health University of Genoa, Genoa16147, Italy

Corresponding author: Giuseppe d'Annunzio, MD, Adjunct Professor, Medical Assistant, Pediatric Clinic and Endocrinology, IRCCS Istituto Giannina Gaslini, Via Gaslini 5, Genoa 16147, Italy. giuseppedannunzio@gaslini.org

Abstract

Endothelial dysfunction (ED) is characterized by an imbalance between vasodilator and vasoconstriction agents. Several pathological conditions clinically diagnosed in childhood and adolescence are characterized by ED and increased risk for early development of microangiopathic and macroangiopathic impairment, in particular type 1 diabetes mellitus (T1DM), T2DM, obesity, metabolic syndromeand pituitary dysfunction associated to various endocrinopathies. More recently insulin resistance following chemotherapy or radiotherapy for tumors, bone marrow transplantation for hematological malignancies (*i.e.*, cancer survivors), or immunosuppressive treatment for solid organ transplantation has been observed. Assessment of ED by means of non-invasive techniques is the gold standard for early ED detection before clinical manifestation. It is aimed to recognize patients at risk and to avoid the development and progression of more serious illnesses. Reactive hyperemia-peripheral artery tonometry is a noninvasive technique to assess peripheral endothelial function by measuring modifications in digital pulse volume during reactive hyperemia, and represents a non-invasive, reproducible and operator-independent tool able to detect precocious ED. This narrative review aimed to provide an overview of the most important papers regarding ED detection by EndoPat 2000 in children and adolescents with different endocrine diseases. A comprehensive search of English language articles was performed in the MEDLINE database without using other search filters except the publication interval between 2005 and 2020.

Key Words: Pediatric diabetes mellitus; Pediatric endocrinopathies; Metabolic syndrome; Cancer survivors; Endothelial dysfunction; Peripheral artery tonometry

manuscript

Specialty type: Pediatrics

Country/Territory of origin: Italy

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

Received: January 29, 2021
Peer-review started: January 30, 2021
First decision: March 16, 2021
Revised: March 30, 2021
Accepted: May 15, 2021
Article in press: May 15, 2021
Published online: June 15, 2021

P-Reviewer: Exbrayat JM, Wang WB
S-Editor: Gong ZM
L-Editor: A
P-Editor: Yuan YY

Core Tip: This narrative review highlights the use of a non-invasive, reproducible and non-operator dependent tool aimed to precocious detection of endothelial dysfunctionin pediatric patients affected by several endocrine diseases, characterized by the risk of vascular impairment. The review also reports a summary of the most important published papers related to the topic.

Citation: La Valle A, Crocco M, Chiarenza DS, Maghnie M, d'Annunzio G. Endothelial impairment evaluation by peripheral arterial tonometry in pediatric endocrinopathies: A narrative review. *World J Diabetes* 2021; 12(6): 810-826
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/810.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.810

INTRODUCTION

Vascular endothelium

Vascular endothelium (VE) is located on the luminal surface of blood vessels and represents a selective, permeable and protective barrier between bloodstream and vascular wall. VE plays several important physiological, paracrine, endocrine and autocrine functions, mainly to assure normalblood fluidity and flow, and to hinder the entry of microbes and other harmful entities, in order to maintain cardiovascular homeostasis. VE also regulates vascular permeability and smooth muscle cell migration, fibrinolysis and thrombosis, platelet and leukocyte adhesion, angiogenesis and vascular tone[1]. Healthy endothelium has also anti-inflammatory properties due to its capability of reaction against hemodynamic changes by production of numerous vasoactive molecules, mainly nitric oxide and prostacyclin[2].

Endothelial dysfunction (ED) is the consequence of mechanical stimuli, like increased endoluminal pressure and shear stress, or metabolic factors like hormones and vasoactive agents. ED is characterized by an imbalance between vasodilator and vasoconstriction agents and is followed by the release of substances aimed to regulate hemostasis, vasomotor activity and inflammation[3]. Moreover damaged endothelium produces agents stimulating either thrombosis, like plasminogen activator inhibitors and von Willebrand factor, and inflammation, like several adhesion molecules, interleukin-6 and ultrasensitive C-reactive protein. ED is one of the most important predictive and pathogenetic mechanism of a broad spectrum of life-threatening conditions, in particular cardiovascular diseases, and represents the primary causative agent of atherosclerosis[4].

Several pathological conditions clinically diagnosed in childhood and adolescence are characterized by ED and increased risk for early development of microangiopathic and macroangiopathic impairment, in particular type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome (MS), and pituitary dysfunction associated to various endocrinopathies. More recently insulin resistance following chemotherapy or radiotherapy for tumors, bone marrow transplantation for hematological malignancies (*i.e.* cancer survivors) or immunosuppressive treatment for or solid organ transplantation has been observed. A new entity, characterized by insulin resistance and type 2 diabetes has been recently defined as new onset diabetes after transplantation (NODAT)[5-16].

In T1DM, chronic hyperglycemia and, more recently defined, glycemic variability impair endothelium function through different mechanisms: oxidative stress, polyol pathway activity, free-radical accumulation, non enzymatic glycosylation of protein, free-radical accumulation[17,18]. All these mechanisms act in different ways and are responsible for the development of various degrees of diabetic microangiopathy, like retinopathy, nephropathy and peripheral neuropathy[19-21]. Thanks to intensive insulin therapy protocols since diagnosis and advances in technological instruments for T1DM management, clinically evident microangiopathy in children and adolescents is almost rarely encountered; on the other hand, subclinical signs of precocious ED can be detected in adolescents, especially in case of poor degree of metabolic control[22,23].

As regards T2DM, obesity, metabolic syndrome and NODAT, insulin resistance (IR) and its metabolic consequences are the most important causative factors of ED. In particular, obesity, insulin resistance and sedentary lifestyle are the main responsible of different co-morbidities related to so called metabolic syndrome, a proinflammatory state that negatively affects endothelial function and characterized by dyslipidemia, hyperuricemia, hypertension[9,24,25].

While in T1DM microangiopathic damage develops during the natural course of the disease, in newly-diagnosed adolescents with T2DM microangiopathic complications, especially nephropathy, have been reported[26]. The early damage negatively affects quality of life and is responsible for morbidity and mortality even at young age[27,28]. In particular, diabetic kidney disease in youth-onset T2DM is reported as a consequence of lower insulin sensitivity, a condition requiring more O_2 consumption and responsible for increased resistance in efferent arterioles[29].

Improved survival rates in childhood cancer, during the past few decades, have increased the population of survivors. Recent estimates allow to hope that in the general population, one every 500-1000 persons will be a childhood long-term cancer survivor. Young cancer survivors and patients who underwent hematopoietic cell transplantation in childhood are at increased risk of MS and cardiovascular disease[30-33]. Total body irradiation, chemotherapy and immunosuppressive agents are causative factors for insulin resistance[33].

Cardiovascular diseases are the most common cause of premature death in Western countries, and in long-term cancer survivors heart diseases are 5-10 times more common than in their siblings[33]. The population of survivors is increasing over time and with it there is the need for a greater understanding of cardiovascular toxicity, which is an overlap of mechanisms directly related to cancer and late effects of oncological therapies[34].

ED may be the first step in the pathogenesis of chronic conditions, such as atherosclerosis, which leads to cardiovascular system disorders including coronary heart disease, hemorrhagic or ischemic stroke, peripheral arterial disease and venous thromboembolism. The major risk factors of endothelial impairment are high-dose chemotherapy with anthracyclines, alkylating agents, vinca alkaloids and total body radiation, especially in younger age. Cancer therapeutic agents may damage endothelial cells and the delicate balance between vasodilating and vasoconstricting substances produced by and acting on endothelial cells. Radiotherapy increases damage of endothelial cells and arterial stiffness through the loss of elastic matrix and the alteration of microvascular structure[30-34].

ED DETECTION

Early ED detection before clinical manifestation is the most important preventive measure, aimed to recognize patients at risk and to avoid the development and progression of more serious illnesses.

Several serum inflammatory markers are available to detect ED, including pro-inflammatory cytokines, like TNF-alpha, IL-1, IL-6, IL-8, IFN-gamma, pigment epithelium derived factors, adipocyte-specific fatty acid-binding protein, lipocalin-2, resistin[4,35,36]. Insulin resistance is associated by a global inflammatory status, characterized in the majority of cases by positivity of several inflammatory markers and is responsible for endothelial impairment leading to angiopathy. At present none of these markers has a prognostic value, and their use in clinical practice remains speculative. Moreover, inflammatory markers detection requires specific laboratories and equipments.

Assessment of ED by means of non-invasive techniques is the gold standard of its detection, and several methods for research purposes have been developed, otherwise their operator dependency and complexity preclude wide use in clinical practice[37].

In particular, reproducibility of a medical test is the consequence of its low intrinsic variability and represents the gold standard for the meaningfulness of the method. Reproducibility depends on the test itself, i.e. operator dependency accuracy of the instrument, and the variability linked to human physiology. For ED detection reproducibility is an important issue, since endothelial function is extremely variable and labile both intra- and inter-subject[38].

To this purpose several diagnostic methods have been developed, based on the following principles: (1) Specific stimuli determine release of NO from VE to mediate its relaxation; and (2) Test measuring ED in different vascular beds. Endothelial vasomotor testing performed in the coronary vascular bed by coronaroangiography

and intracoronary Doppler is the gold standard, however, its invasiveness can raise ethical concerns and is impracticable in research studies[39]. Therefore, alternative vasomotor testing have been proposed in the peripheral circulation, especially in forearm vessels.

Reactive hyperemia-peripheral artery tonometry (RH-PAT) is a non invasive technique to assess peripheral endothelial function by measuring modifications in digital pulse volume during reactive hyperemia, and represents a non-invasive, reproducible and operator-independent tool able to detect precocious ED[40]. Flow mediated dilation has been applied both in adults and adolescents and requires an ultrasound assessment of brachial artery diameter before and after raised shear stress [41,42]. Operator training is required and results may be invalidated by inter-observer variability.

A new automated and less operator-dependent method, named Endo Peripheral Artery Tonometry 2000 (EndoPAT) has been proposed by Itamar Ltd[43]. EndoPAT records endothelium-mediated changes in the digital pulse waveform (PAT signal) using a pair of new modified plethysmographic probes placed on the finger index of each hand[43]. Endothelium-mediated variations of the PAT signal are triggered by inducing a downstream hyperemic response. Hyperemia is elicited by blood flow occlusion through the brachial artery for 5 min using an inflatable cuff on one hand. The response to reactive hyperemia in automatically calculated by the system (Axtec). A PAT ratio is obtained analyzing the pre- and post-occlusion values, and values are normalized to measurements from the controlateral site, considered as control for non-endothelial dependent systemic effects[38] (Figure 1).

To test prospectively the reproducibility and feasibility of EndoPAT, 30 healthy adolescents aged 13 to 19 years were evaluated on 2 different days[38]. The authors concluded that the EndoPAT technique was well tolerated and had excellent reproducibility. On the other hand several factors, mainly pubertal development, may affect microvascular function. To this purpose, Bhangoo *et al*[44] reported that enhancement of the PAT index was positively related to Tanner Stage, probably due to sex steroids influence. Similarly, another study conducted in 94 healthy children and adolescents aimed to evaluate microcirculation by reactive hyperemic index (RHI) reported a positive correlation between RHI and Tanner Stage, age, height, body mass index (BMI), systolic blood pressure values[45]. Conflicting data have been reported about the role of stress and depressive symptoms on endothelial function. Chen *et al*[46] reported adverse effects of negative emotions on peripheral endothelial function, while Olive observed no relationship between ED and self-reported stress or depressive symptoms[47].

AIM OF THE REVIEW

The aim of this narrative review was to provide an overview of the most important papers regarding ED detection through EndoPAT 2000.

A comprehensive search of English language articles was performed in the MEDLINE database without using other search filters except the publication interval between 2005 and 2020. Before 2005 no papers regarding EndoPAT 2000 in pediatric endocrinological and metabolic diseases have been found.

Four authors performed the search, the key words were endothelial dysfunction, Reactive Hyperemia Index, RHI, Peripheral Artery Tonometry, Endo-PAT 2000. For each challenging topic (T1DM, MS and cancer survivors) specific key words were associated and matched with the following terms: Type 1 diabetes mellitus, type 2 diabetes mellitus, obesity, metabolic syndrome, endocrine dysfunction, pituitary, cancer, growth hormone, glucocorticoids, thyroid hormones, estrogens, testosterone, neoplasm, malignancy.

A manual search in the reference lists of most significant papers was also performed. Studies conducted on patients aged more than 18 years or not written in English were excluded. Each study was screened by title and abstract.

For each eligible study we extracted the following data: author, year of publication, design of the study, population studied, control group (if available), RHI results, RHI outcomes. Results are summarized in Tables 1-3.

Table 1 Study characteristics for reactive hyperemic index-Endopat 2000 in different pediatric type 1 diabetes mellitus populations

Ref.	Study design	Aim of study	Population: age mean ± SD or median (range); *n* [F/M]	Control group: age mean ± SD or median (range); *n* [F/M]	RHI result: mean ± SD or median (range)	Outcomes
Mahmud *et al*[51], 2006	RA	Determinate whether a gender contrast in a preclinical stage of atherosclerosis, or endothelial dysfunction, is present in pediatric diabetic patients.	T1DM Children for at least 1 yr, no microalbuminuria or retinopathy: 14.2 ± 1.3, *n* = 20 [8/12]	Healthy children without a family history of hypercholesterolemia: 14.1 ± 1.5, *n* = 20 [8/12]	1.85 ± 0.45 *vs* 1.95 ± 0.32 (diabetic *vs* controls)[c]. 1.61 ± 0.32 *vs* 1.93 ± 0.28 (male diabetic *vs* male controls)[b]. 2.21 ± 0.35 *vs* 1.99 ± 0.38 (female diabetic *vs* female controls)[c]. 1.93 ± 0.28 *vs* 1.99 ± 0.38 (male *vs* female control groups)[c]. 1.61 ± 0.32 *vs* 2.21 ± 0.35 (male *vs* female diabetic groups)[b].	T1DM adolescents males worse RHI compared with similarly aged T1DM females and healthy gender and age matched controls. T1DM females had higher BMI and were more sexually mature.
Haller *et al* [48], 2007	RA	Assess the ability of RHI to serve as a surrogate marker of endothelial dysfunction in children with T1DM.	T1DM Children with disease > 1 yr: 14.4 ± 1.5, *n* = 44 [22/22]	Healthy children, non-smokers and without a family history of medical premature CVD or hyperlipidemia: 14.1 ± 1.5, *n* = 20 [8/12]	1.63 ± 0.5 *vs* 1.95 ± 0.3 (diabetic *vs*controls)[a].	RHI lower in diabetic population. In this study children with T1DM had significantly higher mean systolic BP, mean total cholesterol and mean HDL compared to controls. No significant differences in age, BMI, diastolic BP, LDL or triglycerides were observed between the 2 groups.
Mahmud *et al*[49], 2008	RA	Evaluate the effect of a high-fatmeal on RHI in adolescents with T1DM.	T1DM Children with disease > 2 yr, no retinopathy or nephopathy: 14.6 ± 1.75, *n* = 23 [9/14]	Healthy children: 14.7 ± 1.95, *n* = 23 [9/14]	Pre-meal RHI, T1DM *vs* controls, 1.78 ± 0.4 *vs* 2.06 ± 0.4[a]. Post.meal RHI, T1DM *vs* controls, 1.45 ± 0.3 *vs* 1.71 ± 0.3[a].	RHI lower in diabetic population in a fasting state and after a high-fat meal compared with controls. The change in RHI was similar in the 2 groups.
Palombo *et al*[54], 2011	RA	To compare large artery structure and function indexes, endothelial function and regenerating capacity between T1DM adolescent and healthy age-matched controls. Association of different vascular measures with EPCs, glyco-metabolic control and AGEs, sRAGE and adiponectin levels were searched.	T1DM patients without retinopathy, microalbuminuria and neuropathy, pharmacological treatment (other than insulin). 18 ± 2, *n* = 16 [5/11]	Healthy children: 19 ± 2, *n* = 26 [11/15]	2.0 ± 0.5 *vs* 1.8 ± 0.6 (T1D *vs*controls)[c]. 1.5 ± 0.4 *vs* 2.2 ± 0.8 (T1D with HbA1c 7.5% *vs* T1d with HbA1c < 7.5%)[a].	T1DM adolescents higher central pulse pressure (PP), Augmentation Index (AI), carotid femoral pulse wave velocity, local carotid wave speed, common carotid artery intima-media thickness. RHI reduced only in T1DM patients with 7.5% (P < 0.05). In the overall population, EPCs were an independent determinant of carotid IMT (together with adiponectin), while fasting plasma glucose was an independent determinant of carotid wave speed, AI and central PP.
Pareyn *et al* [50], 2013	CSS	To search a difference in RHI between w T1DM adolescents and controls	T1DM children insulin treated for at least one year: 15.8 (14.4 to 16.6), *n* = 34 [18/16]	Healthy children: 15.5 (13.9 to 16.2, *n* = 25 [13/12]	1.6 (1.3-2.0) *vs* 1.9 (1.7-2.4), children with T1DM *vs* controls[a]. 1.3 (1.3-1.7) *vs* 2.0 (1.7-2.5), female with T1DM *vs* female controls[a]. 1.8 (1.5-2.1) *vs* 1.8 (1.5-2.3), male with T1DM *vs* male controls[c].	RHI lower in T1DM, especially in females. No correlation was seen between RHI and BMI SDS, BP SDS, HbA1c, age, disease duration, TG and Tanner stage.
Scaramuzza *et al*[52], 2015	CS	To evaluate prevalence of early EF, measured by RHI < 1.67 in T1DM cohort, at baseline and after a a 1 yr follow-up	T1DM adolescents with disease duration > 1 yr, Tanner pubertal stage III-V, BMI between 5-95° percentile: 16.2 ± 3.5, *n*	No controls	1.26 ± 0.22 *vs* 2.24 ± 0.48, patients with RHI < 1.67 *vs* patients with RHI > 1.67[b]. At the 1 yr follow-up in 64/73 patients, the rate of	RHI negatively correlates with impaired metabolic control and subclinical signs of autonomic neuropathy, while positively correlates with

			= 73 [25/48]		endothelial dysfunction (81.8%) was even higher than the rate recorded at baseline (76.7%).	regular physical activity. ED progression irrespective of improved metabolic control.
Scaramuzza *et al*[57], 2015	RA	To evaluate the effect of alpha-lipoic acid on ED in T1DM youth, a 6-month, double-blind, randomized controlled trial	T1DM adolescents for at least 1 yr, aged 12-19 yr, insulin requirement 0.5 U/kg/day, blood glucose checks more the 3 times/day, BMI and BP < 95° percentile, no cardiovascular or inflammatory diseases. 16.3 ± 3.4, *n* = 71 [29/42], age at baseline.		3 double-blind study arms: 10000 ORAC antioxidant diet + (-lipoic acid, 1.40 ± 0.68 *vs* 1.72 ± 0.66[a] (baseline *vs* after 6 months). 10 000 ORAC antioxidant diet + placebo, 1.39 ± 0.41 *vs* 1.58 ± 0.40[c] (baseline *vs* after 6 months). Controls, 1.58 ± 0.64 *vs* 1.54 ± 0.42[c].	Positive association between alpha-lipoic acid administration and ED parameters.
Deda *et al* [53], 2018	RA	To evaluate the effect of Vit. D supplementation on EF by RHI measurement	T1DM patients for at least 2 yr and levels of 25-OH-Vit. D < 37.5 nmol/L. 15.7 ± 1.4, *n* = 31 [19/12]	To account for seasonality of RHI testing, a separate cohort of age, sex and T1DM matched controls was tested in spring and in fall (no significant difference was showed)	After a 4.8 ± 1.3 months Vit. D supplementation RHI improved: 1.83 ± 0.42 *vs* 2.02 ± 0.68[a].	Vit.D supplementation associated with EF improvement and reduced expression of urinary inflammatory markers.

[a]*P*<0.05.

[b]*P*<0.005.

[c]*P*>0.05.

AGEs: Serum levels of advanced glycation end products; CS: Cohort study; CSS: Cross sectional study; CVD: Cardiovascular disease; BMI: Body mass index; BP: Blood pressure; ED: Endothelial dysfunction; EF: Endothelial function; EPCs: Endothelial progenitor cells; F: Female; HbA1c: Hemoglobin A1c; LDL: Low-density lipoprotein; M: Male; ORAC: Oxygen radical absorbance capacity units; RA: Research article; RHI: Reactive hyperemia index; SDS: Standard deviation score; sRAGE: Soluble receptors for AGEs; T1DM: Type 1 diabetes mellitus; TG: Triglycerides.

STUDY IN PEDIATRIC PATIENTS WITH DIABETES MELLITUS

We considered 6 studies on EndoPAT use in pediatric patients with T1DM aimed to evaluate precocious ED and 2 other studies aimed to evaluate a diet or drug effect in ED. Five of 8 studies compared RHI between T1DM patients and healthy children. In 3/5 studies RHI was significantly lower in patient group[48-50]; in particular, Pareyn *et al*[50] reported a significantly lower RHI, especially in T1DM females.

Males had a lower BMI and were less sexually mature (3 males were prepubertal, while all the females were post-pubertal)[51]. This might explain the difference reported since it has been described that prepubertal RHI is lower than in a mid-or late pubertal state[44,45]. No other differences between males and females have been reported[51].

T1DM patients evaluated by Haller *et al*[48] had a significantly higher values of systolic blood pressure, total and HDL cholesterol. Blood pressure levels did not always influence RHI in the majority of the studies[51-54], and similar results were found as regards lipid profile[49,51,52-54].

In 1/5 RHI was significantly lower in T1DM males than in females[51].

In the Pareyn cohort 5 patients were overweight or obese, but even after their exclusion RHI was significantly lower in T1DM subjects[50].

In 1/5 RHI was similar between T1DM and control group, although patients had higher central pulse pressure, augmentation index, carotid femoral pulse wave velocity, local carotid wave speed and common carotid artery intima-media thickness [54]. In this study RHI was significantly lower in patients with HbA1c ≥ 7.5% and decreased with diabetes duration.

Excluding the exceptions mentioned above, no differences in age, blood pressure and lipid levels were reported between T1DM and controls. As expected, HbA1c and fasting glucose levels were higher in patients. BMI was not significantly different in most studies[48,49,51], while in some it was not considered[50,54].

In 1/5 studies only T1DM adolescents were included[52]. They were considered to have EDif their RHI was < 1.67 and without ED if their RHI was > 1.67. RHI was significantly lower in 76.7% of patients, and after a one year follow-up increased to 81.8%. The authors reported that RHI was significantly correlated to HbA1c levels (both at baseline, and measured during the follow-up period), to subclinical signs of

Table 2 EndoPat 2000 in pediatric population with metabolic syndrome

Ref.	Design	Aim of the study	Population: age in years; mean ± SD or median (range)	Control group: age in years; mean ± SD or median (range)	RHI reported in arbitrary units. If RHI not specified, we reported p trend or positive/negative relation with parameters examined	RHI outcomes
Dongui *et al* [58], 2019	QRS	Impact of diet and exercise on microvascular function	Sedentary OB Age 12-18, *n* = 57 [F/M = 0/57]	Healthy NW Age 12-20, *n* = 10 [F/M 0/10]	OB 1.43 (0.35) *vs* CG 1.67 (0.36)[a]. After exercise OB *vs* CG[a]. OB Pre-exercise *vs* Post-Exercise[a].	RHI higher in CG. In OB RHI improved after 6 wk of diet and exercise.
Pareyn A *et al* [59], 2015	CSS	Assessment of EF in OB/OW adolescents	OW/OB Age 14.7, *n* = 27 [F/M 11/16]	NW Age 15.5, *n* = 25 [F/M 13/12]	NW 1.88 (1.7-2.4) *vs* OW 1.5 (1.3-1.9)[a]. Positively with age[a] and tanner stage[a]. Negatively with diastolic BP[a]. With BGL, insulin lipid profile[c].	RHI lower in OB/OW adolescents. RHI improved with age and Tanner stage. RHI decreased with higher diastolic BP. RHI not related with lipid, IR, BGL and gender. RHI inversely related with baseline pulse amplitude.
Agarwal *et al* [60], 2013	CSS	Assessment of EF in OB/NW adolescents	OB Age 15.3 (0.4) years, *n* = 37 [F/M 26/11]	NW Age 14.9 (0.6), *n* = 14 [F/M 9/5]	OB 1.7 (0) *vs* NW 1.9 (0.1)[a]. OB IGR 1.63[a]. Other values reported like p trend.	RHI lower in obese adolescents. RHI negatively related with BMI, WC, BGL, HOMA-IR, Leptin, TNF, hs-CRP. No relationship with lipid profile and BP.
Mahmud *et al* [61], 2009	RA	Evaluation of EF in OB adolescents with impaired IS	OB with HOMA-IR 5.4 Age 13.4 (1.7), *n* = 26 [F/M 10/16]	NW, healthyAge 14 (1.4), *n* = 51 [F/M 21/30]	OB 1.5 (0.4) *vs* NW 2 (0.4)[b]. Other values reported like p trend.	EF lower in OB and negatively related with adiposity, TG, LDL and Tot-Chol. RHI improved with age. RHI not correlated with Leptin, IR or gender.
Tomsa *et al* [62], 2016	CSS	Comparing EF to body fat, IS, BGL and CIM in dysglicemic and OW adolescents	OW with NGT, *n* = 25, OW with IGT *n* = 19, OW with T2D but HB1Ac < 8% *n* = 16; Age 15.5 (0.2) Total *n* = 60 [F/M 37/23]	NW Age 15.5 (0.2), *n* = 21 [F/M 9/12]	BMI 30.91.2 (0) *vs* BMI 30.41.5 (0) *vs* BMI 26.72.0 (0)p trend[a]. Negatively with WC, BGL, TNF, PAI[a],leptin[b]. Positively with age and insulin sensitivity[a]. BP, lipid profile[c]. For exact values see reference.	RHI lower in OB and T2DM. RHI negatively related with percentage body fat, WC, Leptin, TNF-alpha, BGL. RHI positively related with age and. RHI not related with BP and lipid profile.
Del Ry *et al* [63], 2016	RA	C-type Natriuretic Peptide in OW, OB and NW. Relation with RHI and other endothelial markers	OW AGE 12.8 (1.6) *n* = 10; [F/M 5/5]. OB, G 3.5 (1), AGE 12.8 (1.6) *n* = 45; [F/M19/26].	NW, AGE 12.8 (1.4) *n* = 27; [F/M 14/13]	NW 2.1 (0.2) *vs* OW 1.6 (0.4)[a]. NW *vs* OB 1.4 (0.3)[b]. Negatively with CNP[b]. Exact values non reported.	RHI was significantly lower in OW/OB. CNP negatively related with RHI.
Del Ry *et al* [64], 2020	RA	Natriuretic peptide network in normal weight and obese adolescents, its relation with RHI.	Primary OB Not diabetic, Age 13.3 (0.5) *n* = 16; [F/M8/8].	NW, Age 14.3 (0.4) *n* = 24; [F/M14/10].	NW 2.1 (0) *vs* OB 1.4 (0)[b]. Negatively with CNP, hs CRP, diastolic BP[b]. Exact values non reported.	RHI significantly lower in OB.RHI negatively related with hs-CRP, CNP, diastolic BP, fat mass and A1C.
Singh *et al* [65], 2017	RA	Relation between EF and urinary markers	OW and OB Age 13.8 (2.4) *n* = 43; [F/M 23/20]	Healthy NW Age 13.9 (2) *n* = 20; [F/M 8/12]	NW 1.6 (0.1) *vs* OW 1.66 (0.1)[c] and OB 1.67 (0.1)[c]. NW girls 1.9 *vs* NW boys 1.25[b].	No correlation between RHI, BMI and urinary markers. RHI higher in NW female adolescents
Czippelova *et al*[66], 2019	RA	Assessment of EF in different systemic vascular resistances. Comparing EF to Cardio Ankle Vascular Index	OB No DM or HBP Age 16.4 (2.7) *n* = 29 [F/M 14/15]	NW Age 16.5 (2.6) *n* = 29 [F/M NR]	NW 1.45 (0.3) *vs* OB 1.4 (0.3)[c]. Positively with SVR[a].	No difference between RHI in OB and CG RHI was influenced by vascular tone and resistance. RHI in OB positively related with SVR.
Kochummen *et al*[24], 2019	CSS	Evaluation of EF in OB with normal BGL comparing to NW with T1DM1 and OB with T2DM	NW with DM1 and OB DM2 Age 12.7 (3.8) *n* = 41 [F/M 25/16]	OB with normal BGL, BP and lipid profile. Age 12.8 (2.7) *n* = 17 [F/M 9/8]	A1C > 10% 1.2 (0.2) *vs* A1C < 10% 1.7 (0.6)[a]. Negatively with A1C[a]. DM 1.4 (0.5) *vs* obese 1.4 (0.3)[c]. T1D 1.4 (0.5) *vs* T2D1.5 (0.5)[c]. Female 1.5 (0.5) *vs* male 1.3 (0.4)[a].	RHI lower in poorly controlled DM. RHI negatively related with A1C. RHI similar between OB and NW with DM and between DM1 and DM2. RHI lower in males especially in OB without

						DM.
Bruyndonckx *et al*[67], 2014	CSS	Evaluation of EF and correlation with CVRF in children	OB Age 15.2 (1.4) *n* = 57	NW Age 15.5 (1.5) *n* = 30	NW 2 (0.6) *vs* OB 2.2 (0.7)[c]. Lipid, HOMA-IR, BP, hsCRP[c].	RHI not related with BMI, HOMA-IR, BP, lipid or hsCRP. RHI not homogenous with "Time to peak".
Tryggestad *et al*[68], 2012	RA	Evaluation of vascular function in OB and NW children	OB Age 13.9 (2.5) *n* = 62 (F/M 32/30)	NW Age 13.3 (3) *n* = 61 (F/M 30/31)	OB *vs*CG[c]. Exact values reported for age and BMI (see reference). Age group 8-12 yr: 1.6-2.0[a]. Age group 13-18 yr: 2.0-2.5[a].	RHI similar in OB and CG. RHI improved 0.07 for each year of age in CG. RHI was reduced in older OB. RHI not related with BP and lipid profile.
Fusco *et al* [69], 2020	RA	Assessment of precocious microvascular dysfunction in OB adolescents	OB Age 14.1 (2.5), *n* = 22 [F/M 13/9]	NW Age 15.1 (1.5) *n* = 24 [F/M 11/13]	OB 1.8 (0.6) *vs* CG1.9 (0.5)[c].	RHI not different between CG and OB. RHI not correlated with LDF (that is impaired in OB).
Bacha *et al* [74], 2017	CSS	Comparing EF in hispanic adolescents with and without NAFLD	OW with pre diabetes or T2DM with NAFLD Age 15.2 (0.5) *n* = 23 [F/M 12/11]	OW with pre-diabetes or TD2 without NAFLD Age 15.7 (0.4) *n* = 13 [F/M 3/10]	NAFLD 1.4 (0) *vs* CG 1.7 (0)[b].	Hepatic fat and AST/ALT levels were inversely related with RHI.

[a]*P*< 0.05.
[b]*P*< 0.005.
[c]*P*> 0.05.
A1C: Glycosylated hemoglobin; AE: Anti-epileptic drugs; CIM: Circulating inflammatory markers; CNP: C-type natriuretic peptide; CSS: Cross sectional study; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; CVR: Cardio vascular risk; CVRF: Cardio vascular risk factors; EF: Endothelial function; HBP: High blood pressure; IGT: Impaired glucose tolerance; IR: Insulin resistance; IS: Insulin sensitivity; LDF: Laser Doppler flowmetry; NW: Normal weight; OB: Obese > 95'cc; OS: Oral steroids; OW: overweight > 85' cc; NGT: Normal Glucose Tolerance; PM: Psychiatric medications; QRS: Quasi randomized study; RA: Research Article; SVR: Systemic vascular resistance; TG: Triglycerides; Tot-Chol: Total cholesterol; RHI: Reactive hyperemic index; WC: Waist circumference.

autonomic neuropathy and to physical activity[52]. However, RHI did not correlate to gender, carotid intima media thickness, insulin requirement, dietary habits and body composition[52].

Inter-individual values of RHI in some studies were not correlated to Hb1Ac[48,50,51] age and disease duration[50-52], BMI or lipid profile[48,50-52,54], blood pressure [48,51,52], fasting glucose levels[48-54], or pubertal stage[51]. Pareyn *et al*[50] described a weak correlation between inter-individual RHI and Low Density Lipoprotein (LDL), however this correlation was counterintuitive and might be a type I error.

An interesting study by Heier *et al*[55] was not included in Table 1, since mean age was 20.8 ± 1.8 years. ARHI < 1.67 was reported in 30.4% of patients with diabetes and in 21.4% of controls. This might indicate that the cut-off reported by the Mayo study was not ideal for assessing cardio vascular disease (CVD).

A double blind, randomized, placebo-controlled trial (RCT) was conducted in 443 T1DM adolescents at high-risk of CVD and renal complications, defined as albumin-creatinine ratio (ACR) in the upper tertile of range[56]. The aim of this study was to evaluate the effect of ACE inhibitor, statin and combinations of bothinterventions or placebo. In addition a parallel observation cohort of T1DM subjects defined as low-risk of complications (ACR in lower and middle tertiles) was compared with the untreated group. To evaluate ED, RHI in 158 patients from the RCT and 215 patients from the observation cohort was evaluated[56]. No differences in RHI were observed between high- and low-risk CVD participants in the observational study[56]. Neither ACE nor statin use had any effect on RHI in RCT. During the follow-up RHI increased. An improvement of microvascular function possibly due to stature and pubertal development might explain this result[44,56]. The authors reported that adjusting RHI for body mass normalized RHI to baseline values[56].

1/8 studies investigated the effect of Vit. D supplementation in ED in T1DM patients with 25-OH-VitD levels < 37.5 nmol/L[53]. An improvement of RHI after 4.8 ± 1.3 mo follow-up has been reported.

1/8 studies used EndoPAT to evaluate the effect of an alpha-lipoic acid and antioxidant diet in adolescents with T1DM in a double blind trial, and an improvement in the group treated with 10000 ORAC antioxidant diet +-lipoic acid has been reported[57].

Table 3 Study characteristics for reactive hyperemic index-Endopat 2000 in different pediatric endocrine populations

Ref.	Study design	Aim of study	Population: age mean ± SD or median (range); *n* [F/M]	Control group: age mean ± SD or median (range); *n* [F/M]	RHI result: mean (SD)	Outcomes
Bhangoo *et al*[44], 2011	CSS	Relation of puberty and sex steroids with endothelial function	Healthy population: Tanner I: 12.1 (0.6), *n* = 21 [19/2] Tanner II-III: 12.7 (0.7), *n* = 35 [21/14] Tanner IV-V: 13 (0.7), *n* = 33 [22/11]		Tanner I 1.46 (0.44) *vs* Tanner II-III 1.71 (0.35)[a]. Tanner I 1.46 (0.44) *vs* IV-V 1.92 (0.38)[b]. Tanner II-III value n.avs IV-V value NA[a]. F Tanner II-III 1.66 (0.38) *vs* F IV-V 1.91 (0.29)[a]. M Tanner I 1.41 (0.35) *vs* M Tanner II-III 1.78 (0.30)[b]. M Tanner I *vs* M Tanner IV-V 1.93 (0.67)[a].	PAT index positively related with estradiol, DHEAS levels and age.
O'Gorman *et al*[94], 2012	CCS	Evaluation of EF in TS, and HC.	Turner syndrome: 13.5 (2.4), *n* = 15 [15/0]. Turner syndrome: *GH-untreated* 14.3 (2.4), *n* = 8. Turner syndrome: *GH-treated* 12.7 (2), *n* = 7.	Healthy children (HC) 14.3 (1.7), *n* = 15 [15/0]	Turner syndrome: 1.64 (0.34) *vs* HC 2.08 (0.32)[b]. Turner syndrome: *GH-untreated* 1.44 (0.26) *vs* *GH-treated* 1.86 (0.28)[a].	PAT index lower in TS indicating impaired EF compared with HC.GH may protect endothelial function in TS.
Ruble *et al* [78], 2015	CCS	Evaluation of RHI in ALL survivors, compared with HS.	ALL survivors: (0.9), *n* = 16 [8/8]. HS:13.8 (0.9), *n* = 16 [6/10].	HS: 14.3 (1.7), *n* = 15 [15/0]	ALL survivors 1.54 (0.38) *vs* HS 1.77 (0.41)[a].	Poorer vascular health ALL survivors.
Blair *et al* [77], 2014	RCCT	Evaluation of flavanoid-rich purple grape juice (compared in RCCT with clear apple juice) on endothelial function, markers of oxidative stress and inflammation in cancer survivors.	Cancer survivors (hematopoietic malignancy 50%, solid tumor 50%) 16.4 (13.7–17.2), *n* = 24 [17/7]		Cancer survivors. Before apple juice 1.57 (0.36) *vs* before grape juice 1.75 (0.52). After apple juice 1.83 (0.47) *vs* after grape juice 1.75 (0.39). Before grape juice 1.57 (0.52) *vs* after grape juice 1.75 (0.39).	After four weeks of daily consumption of flavanoid-rich purple grape juice, no measurable change in vascular function in young cancer survivors.

[a]*P*<0.05.
[b]*P*<0.005.
CSS: Cross sectional study; CCS: Case control study; F: Female; GH: Growth hormone; HC: Healthy controls; HS: Healthy siblings; ALL: Acute lymphoid leukemia; M: Male; NA: Not available; RHI: Reactive hyperemic index; RCCT: Randomized controlled crossover trial.

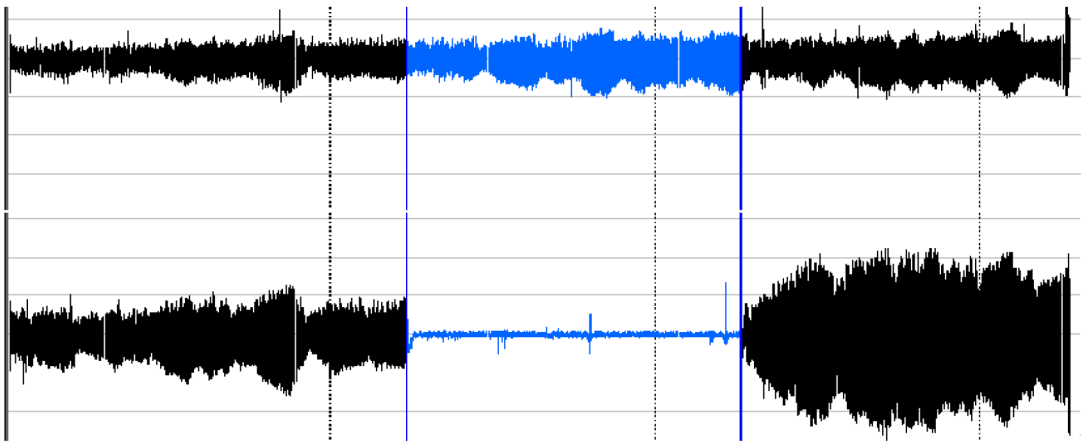

Figure 1 Patient with type 1 diabetes mellitus, the upper highlighted line shows normal flow in the non-occluded right harm. The lower highlighted line shows total occlusion in the left occluded harm. The picture shows a non pathologic post-occlusion dilation with RHI 1.8, despite this value is lower than mean values for young healthy boys.

Most of the studies reported an ED in young adults with diabetes mellitus as compared with healthy controls despite an absence of clinical manifestation of CVD, other co-morbidities (*e.g.*, retinopathy or microalbuminuria) or traditional cardiovascular risk. RHI correlation with glycemic control is unclear; in some studies RHI was negatively related with HbA1c or diabetes duration. The evaluation with

EndoPAT in pediatric age is likely difficult due to the physiological development through puberty. Maybe EndoPAT might be helpful in evaluating the effect of some external interventions (*e.g.*, medication or diet).

STUDY IN PATIENTS WITH METABOLIC SYNDROME

We considered 14 studies describing the use of EndoPAT 2000 for the assessment of ED in adolescents with MS, reporting conflicting results. The following parameters were considered: BMI, T1DM and T2DM, gender, pubertal stage, age, polycystic ovary syndrome (PCOS), blood pressure (BP) values, non alcoholic fatty liver disease (NAFLD), obstructive sleep apnea (OSA), lipid profile, IR and insulin sensitivity (IS) indexes, plasma glucose (PG) levels, inflammatory markers (Urinary Markers, CNP, PAI, Adiponectin, Resistin, micro RNA-126, VCAM-1, E-Selectin, I-CAM, saturated fatty acids). Results of the most important studies are reported in Table 2.

As regards BMI, in 7/14 studies RHI was significantly lower in obese adolescents [58-64]. In particular, Donghui *et al*[58] evaluated obese males and showed improvement of RHI after six weeks of diet and intensive training. They concluded that EndoPAT 2000 is a useful tool to evaluate vascular endothelial function[58]. In Mahmud *et al*[61] RHI was negatively related with BMI and lipid profile, but not with IR indexes and adipocytokine levels. In Tomsa *et al*[62] waist circumference was the main determinant of ED (P = 0.0004). However, in 6/14 studies RHI did not correlate with BMI[24,65-69]. In the study of Czippelova *et al*[66] RHI was not influenced by BMI, but was related with systemic vascular resistance. In Bruyndonckx *et al*[67] RHI was similar in obese and controls, while time to peak was significantly lower in obese. Similar results were reported by Hudgins *et al*[70]. In Fusco *et al*[69] RHI was similar in obese and controls. They also evaluated laser Doppler flowmetry (LDF) as a marker of precocious microvascular damage, and showed LDF lower levels in obese as compared to controls. They concluded that precocious ED in childhood obesity cannot be evaluated by RHI[69].

To our knowledge, 2/14 studies compared RHI to T1DM and T2DM[62,67]. In particular in Kochummen *et al*[24] mean RHI in obese adolescents without diabetes was similar to T1DM and T2DM patients. RHI was lower if compared with healthy controls obtained by other authors, and decreased of 0.09 for each 1% increase of HbA1c, but no difference was observed between patients with T1DM and T2DM. In Tomsa *et al*[62] RHI was higher if HbA1c was less than 5.5%, and was lower in T2DM obese.

In 2/14 studies RHI was higher in female adolescents. In particular RHI was higher only in girls belonging to the control group[24,65]. Conversely, RHI was not different between boys and girls[61]. RHI was positively related with Tanner Stage in one study [59], while another study did not report difference between pre-pubertal and pubertal children[24].

In 4/14 studies RHI improved with age[59,61,62,68]. In Tryggestad *et al*[68] RHI improved of 0.07 units for each year of age in control group only, but not in obese subjects. In two studies RHI decreased with age in obese adolescents, especially older than 15 years, maintaining this trend in adulthood. It has been suggested that in obese subjects RHI is not precociously impaired, but more time is needed to establish endothelial damage. In 1/14 study RHI was similar among different age groups[24]. Another study in pediatric population showed RHI improvement following increasing height and stage[45].

Lowenstein *et al*[71] found that RHI was lower in women with PCOS. The study did not include pediatric patients, however, it is important since girls with MS are at risk of developing PCOS. Other authors showed that EF was similar before and after 3 mo of metformin treatment for PCOS, suggesting that longer periods of metformin are needed to evaluate its positive effect on endothelial function[72,73].

In 5/14 studies RHI was not related with blood pressure values[60-62,67,68], while in 2/14 studies RHI was inversely related with blood pressure, but only with the diastolic ones[59,64].

Only in the study of Bacha *et al*[74] RHI was not compared with BMI. In this study RHI was negatively related with NAFLD, liver enzyme levels and liver-fat deposition. Moreover, RHI was negatively associated with augmentation index, which was higherin patients with NAFLD[74]. Kheirandish-Gozalet al[75] evaluated RHI twice a day (morning and evening) and showed that RHI was lower in morning than in evening, and related with OSA severity score. They concluded that RHI was significantly lower than in controls and confirmed the usefulness of EndoPAT

instrument. Only in 1/14 studies RHI was negatively related with total cholesterol (P < 0.01), triglycerides (P <0.04), and LDL (P = 0.02) levels, but not with HDL levels[61]. In 5/14 studies RHI was not related with lipid profile[59,60,62,67,68]. On 1/14 studies RHI was negatively related with IR indexes[60] and in another study RHI was positively related with insulin sensitivity index[62]. In other 4/14 studies RHI was not statistically related with IR indexes[59,61,67,68].

In 2/14 studies RHI was not related with fasting PG[59,61] while in 4/14 studies RHI was negatively related with PG[24,60,62,64].

In 2/14 studies RHI was inversely related with Leptin and TNF-alpha levels[60,62], and in 3/14 studies RHI was inversely related with HSCRP[60,63,64]. As for other aspects of metabolic syndrome these results were not homogenous. In 3/14 studies RHI was not related with HSCRP[65,67,68], and in 2/14 studies RHI was not related with Leptin levels[61,65]. In some studies RHI was compared to other inflammatory markers less used to assess endothelial function[58,61-65,74,76]. None of these reported LnRHI. Only one study (not in table) reported "Peak response" instead of RHI[70].

Chen *et al*[76] studied RHI and its relationship with HOmeostatic Model Assessment of Insulin Resistance (HOMA-IR). RHI was evaluated in 257 healthy adolescents (138 F/119M) and was negatively related with HOMA-IR (P = 0.001), and not with gender, lipid profile, BP values[75].

In 3/14 studies limitations were not reported[58,70,65]. Mahmud *et al*[61] evaluated controls recruited from other studies, and IR and adipocytokine levels from control group are not known. Tomsa *et al*[62] evaluated patients with T2DM during metformin therapy which is recognized to influence endothelial function. In 5/14 studies patients' samples were small, including 41, 45, 37, 27 and 29 participants, respectively[24,59,60,64,66]. Bacha *et al*[74] evaluated Hispanic population, while Mahmud *et al*[61] enrolled Caucasian adolescents. In 1/14 study are reported limitations about the reliability of RH-PAT[59]. Pareyn *et al*[59] concluded that an unequivocal theory about the underlying mechanism is lacking. There is some uncertainty about the relative importance of endothelium dependent and independent vasodilatation factors in the RH-PAT response. Vascular bed and RH-PAT are highly responsive to sympathetic tone and to emotional responses like anger, depression, anxiety[59].

ENDOTHELIAL FUNCTION IN CANCER SURVIVORS

To our knowledge two studies analyzed ED by Reactive Hyperemia Index – Peripheral Artery Tonometry (RHI-PAT) in childhood cancer survivors (Table 3).

Blair *et al*[77] examined microvascular endothelial function in childhood cancer survivors off therapy for more than three years; 21 out of 24 participants received cardiovascular toxic chemotherapies (anthracyclines or platinum agents), and/or radiation. They showed low/borderline RHI-PAT value without measurable change in vascular function after four weeks of supplementing meals with flavonoid-rich purple grape juice.

Ruble *et al*[78] compared 16 acute lymphoblastic leukemia (ALL) survivors from one to ten years off therapy, with 16 healthy siblings matched by gender and age. All but one survivor has been treatedwith anthracyclines with a mean cumulative dose of 148 mg/m^2 and nearly one third has undergone cranial radiation. In this study, adolescent ALL survivors had significant lower RHI-PAT (Table 3), despite similar cardiovascular clinical risk factors with control group, including BMI, blood pressure values and waist to height ratio (marker of central adiposity), marker of fitness (assessed by Six Minute Walk Test). However, more survivors than siblings resulted overweight or obese (44% *vs* 31%). No data are available about lipid profiles, despite previous study on long-term survivors[79,80] have suggested that abnormal triglycerides levels contribute to macrovascular ED measured by endothelial dependent ultrasound flow-mediated dilation (% of change in brachial artery diameter after 5 min of occlusion).

Little is known about the incidence and predisposing factors of ED after radiotherapy. Dengel *et al*[79] found no difference in endothelial function in survivors who underwent cranial radiotherapy in addition to chemotherapy compared with control group of similar gender, age, and weight. In a subsequent cross-sectional study Zelcer *et al*[81] showed ED in 13 Hodgkin lymphoma young adult survivors who had received mediastinic radiotherapy compared with healthy gender- and age-matched controls, as evidenced by lower RHI-PAT (1.67 ± 0.39 *vs* 2.03 ± 0.37, P <0.01). No correlation between PAT scores in controls and Hodgkin lymphoma survivors was found for any of the classic cardiovascular risk factors (BMI, systolic and diastolic

blood pressure, serum LDL, HDL, and triglycerides levels). Vatanen *et al*[82] demonstrated that childhood cancer survivors treated with total body irradiation (TBI) develop indirect signs of endothelial damage during adulthood, including decreased arterial lumen size and an increased carotid intima-media thickness, but no pediatric studies have used RHI-PAT to detect endothelial function after TBI.

ENDOTHELIAL IMPAIRMENT IN PITUITARY DYSFUNCTION

Endothelial function testing has received growing interest as early marker of cardiovascular risk in pediatric endocrine diseases. During puberty, a complex interplay between metabolic and hormonal factors may affect endothelial function, the hypothalamus pituitary axis plays a central role in this complex network, although the exact mechanisms are not completely understood[42]. Estrogens can potentially enhance the endothelial-dependent flow mediated vasodilatation *via* the production of NO (nitric oxide) by endothelial NO synthase enzyme. In several in vitro animal models, it was shown that endothelial NO production and vasodilatation increases during puberty. In two different clinical studies, significant correlations with RHI-PAT were observed for pubertal stage[83,84].

Bhangoo *et al*[44] demonstrated that an increase in the RHI with pubertal advancement was related to an increase in sex hormones. In a healthy population of 89 children and adolescents, pubertal staging was based on ultrasensitive estrogen assays. A positive correlation between RHI-PAT and steroid hormone levels was found.

Radtke *et al*[45] confirmed this observation in two separate prospective cross-sectional studies. These included 112 healthy, normal weight and normotensive 10–16 year old children and adolescents, classified using a validated self-assessment tool, according to Tanner Stage, in 3 groups: prepubertal, (Tanner I); mid-puberty (Tanner II-III); late puberty (Tanner IV-V). Prepubertal children had a significantly lower RHI-PAT as compared to mid-puberty or late puberty groups (Table 3). In contrast to the results of Bhangoo *et al*[44], they found significant negative correlations between RHI-PAT and both stature (r = 0.553; P< 0.001) and BMI (r = 0.309; P = 0.001). Significant negative correlations were also observed between RHI-PAT and both age (r = 0.567; P< 0.001) and systolic blood pressure levels (r = 0.494; P< 0.001). However, in stepwise regression analysis pubertal status was the only independent predictor of ED (R^2 = 0.242; β = 0.492; P< 0.001). In both studies an important limitation lies in the methods used to determine the Tanner Stage, since pubertal stage was assessed by a self-administered questionnaire[85] or based on ultrasensitive serum estradiol levels, rather by physical examination.

The reduction of nitric acid (NO) also occurs in patients with growth hormone deficiency (GHD) due to reduction of local IGF-I, which causes dependent endothelial vasodilatation through the stimulation of NO production[86]. Moreover, GHD may contribute to the ED by increasing reactive oxygen species (ROS)[87].

On the other hand, there are studies showing an impairment of endothelial function in endocrine disease caused by hypersecretion of GH[88]. Although low level of IGF-1 is associated with ED, high IGF-1 is also related with increased endothelial impairment and cardiovascular disease[89,90]. In acromegaly, the mechanisms underlying this effect could be an imbalance between endothelium derived vasodilators (particularly NO) and ROS, together with increase of blood pressure, insulin and lipid profile values[91,92]. Along with these risk factors, GH and insulin-like growth factor-1 (IGF-1) can directly cause changes in macrovascular structures by vasoconstriction IGF-1 mediated *via* the nitric oxide synthesis or damage to vascular smooth muscle cells[93].

Despite several studies have analyzed the role of GH deficiency or hypersecretion in ED in adulthood, only one study has focused on pediatric age. In a cross-sectional case-control study, O'Gorman *et al*[94] found that adolescents with Turner syndrome (TS) had impaired endothelial function compared to healthy age-, gender- and BMI-matched controls. In TS GH therapy may protect endothelial function. Indeed, RHI-PAT scores were higher in girls receiving GH therapy than in those not receiving it (Table 3) and there was no significant difference in RHI-PAT scores between TS receiving GH therapy and control population (1.86 ± 0.28 *vs* 2.08 ± 0.32,respectively, P = 0.14). In the same study, RHI-PAT scores inTS population did not vary with estrogen replacement therapy [1.56 ± 0.30 with estrogen replacement (n = 6) *vs* 1.69 ± 0.37 without estrogen replacement (n = 9), P =0.64]. The study is limited by several factors: the small number of patients, the lack of data about pubertal status, independent cardiovascular risk factors in TS patients (including 1 patient with a history of

coarctation repair).

To our knowledge, no studies have investigated the possible relationships between ED and pituitary disorders in pediatric age.

CONCLUSION

Results from data on healthy populations suggest that a low RHI-PAT in children and adolescents is more likely to reflect juvenile microvascular response due to immature endothelial function rather than pathological dysfunction. Therefore, the risk thresholds established for the adult population cannot be used without necessary considerations in pediatric age. GH therapy may restore ED associated with growth hormone deficiency and may offer endothelial protection in girls with TS. IGF1 is considered as a key molecule in the pathogenesis of microvascular damage. There is a need of new studies to evaluate the endothelial function in other pediatric hypothalamus-pituitary axis disorders.

Oncological therapies may impair endothelial function and microvascular structure. Several chemotherapies and radiotherapy are risk factors for cardiovascular disorders in childhood cancer survivors. Therefore, it is essential to monitor microvascular endothelial function and long-term follow-up of survivors is recommended. Assessment of ED in cancer survivors, by a non-invasive and easily reproducible methodology as RHI-PAT, may have a role in the early identification of late effects in patients at high risk of cardiovascular events who could benefit from cardioprotective pharmacological interventions. However, there are no established reference values in children, and it is unknown the reliability of RHI-PAT to predicting adverse cardiovascular events. Therefore, due to lack of reference ranges, evaluation of PAT remains a research tool.

REFERENCES

1 **Godo S**, Shimokawa H. Endothelial functions. *Arterioscler Thromb Vasc Biol* 2017; **37**: e108-e114 [PMID: 28835487 DOI: 10.1161/ATVBAHA.117.309813]

2 **Shimokawa H**, Godo S. Diverse functions of endothelial NO synthases system: NO and EDH. *J Cardiovasc Pharmacol* 2016; **67**: 361-366 [PMID: 26647119 DOI: 10.1097/FJC.0000000000000348]

3 **Taylor MS**, Choi CS, Bayazid L, Glosemeyer KE, Baker CCP, Weber DS. Changes in vascular reactivity and endothelial Ca^{2+} dynamics with chronic low flow. *Microcirculation* 2017; **24** [PMID: 28106317 DOI: 10.1111/micc.12354]

4 **Leite AR**, Borges-Canha M, Cardoso R, Neves JS, Castro-Ferreira R, Leite-Moreira A. Novel biomarkers for evaluation of endothelial dysfunction. *Angiology* 2020; **71**: 397-410 [PMID: 32077315 DOI: 10.1177/0003319720903586]

5 **Tsukahara H**. Biomarkers for oxidative stress: clinical application in pediatric medicine. *Curr Med Chem* 2007; **14**: 339-351 [PMID: 17305536 DOI: 10.2174/092986707779941177]

6 **Anık A**, Çelik E, Çevik Ö, Ünüvar T, Anık A. The relation of serum endocan and soluble endoglin levels with metabolic control in children and adolescents with type 1 diabetes mellitus. *J Pediatr Endocrinol Metab* 2020 [PMID: 32697760 DOI: 10.1515/jpem-2020-0146]

7 **Domingueti CP**, Dusse LM, Carvalho Md, de Sousa LP, Gomes KB, Fernandes AP. Diabetes mellitus: The linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *J Diabetes Complications* 2016; **30**: 738-745 [PMID: 26781070 DOI: 10.1016/j.jdiacomp.2015.12.018]

8 **Hoffman RP**. Vascular endothelial dysfunction and nutritional compounds in early type 1 diabetes. *Curr Diabetes Rev* 2014; **10**: 201-207 [PMID: 24925525 DOI: 10.2174/1573399810666140613124326]

9 **Shah AS**, Urbina EM. Vascular and endothelial function in youth with type 2 diabetes mellitus. *Curr Diab Rep* 2017; **17**: 36 [PMID: 28432570 DOI: 10.1007/s11892-017-0869-0]

10 **Vaughan TB**, Ovalle F, Moreland E. Vascular disease in paediatric type 2 diabetes: the state of the art. *Diab Vasc Dis Res* 2007; **4**: 297-304 [PMID: 18158699 DOI: 10.3132/dvdr.2007.056]

11 **Herouvi D**, Karanasios E, Karayianni C, Karavanaki K. Cardiovascular disease in childhood: the role of obesity. *Eur J Pediatr* 2013; **172**: 721-732 [PMID: 23340698 DOI: 10.1007/s00431-013-1932-8]

12 **Short KR**, Blackett PR, Gardner AW, Copeland KC. Vascular health in children and adolescents: effects of obesity and diabetes. *Vasc Health Risk Manag* 2009; **5**: 973-990 [PMID: 19997578 DOI: 10.2147/VHRM.S7116]

13 **Halici I**, Palabiyik SS, Guducu-Tufekci F, Ozbek-Bilgin A, Cayir A. Endothelial dysfunction biomarker, endothelial cell-specific molecule-1, and pediatric metabolic syndrome. *Pediatr Int* 2016; **58**: 1124-1129 [PMID: 27011259 DOI: 10.1111/ped.12989]

14 **Brouwer CA**, Postma A, Hooimeijer HL, Smit AJ, Vonk JM, van Roon AM, van den Berg MP,

Dolsma WV, Lefrandt JD, Bink-Boelkens MT, Zwart N, de Vries EG, Tissing WJ, Gietema JA. Endothelial damage in long-term survivors of childhood cancer. *J Clin Oncol* 2013; **31**: 3906-3913 [PMID: 24062395 DOI: 10.1200/JCO.2012.46.6086]

15 **Almici C**, Skert C, Bruno B, Bianchetti A, Verardi R, Di Palma A, Neva A, Braga S, Piccinelli G, Piovani G, Malagola M, Bernardi S, Giaccone L, Brunello L, Festuccia M, Baeten K, Russo D, Marini M. Circulating endothelial cell count: a reliable marker of endothelial damage in patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2017; **52**: 1637-1642 [PMID: 28892085 DOI: 10.1038/bmt.2017.194]

16 **Jodele S**, Dandoy CE, Myers KC, El-Bietar J, Nelson A, Wallace G, Laskin BL. New approaches in the diagnosis, pathophysiology, and treatment of pediatric hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Transfus Apher Sci* 2016; **54**: 181-190 [PMID: 27156964 DOI: 10.1016/j.transci.2016.04.007]

17 **Varvarovská J**, Racek J, Stetina R, Sýkora J, Pomahacová R, Rusavý Z, Lacigová S, Trefil L, Siala K, Stozický F. Aspects of oxidative stress in children with type 1 diabetes mellitus. *Biomed Pharmacother* 2004; **58**: 539-545 [PMID: 15589060 DOI: 10.1016/j.biopha.2004.09.011]

18 **Kostolanská J**, Jakus V, Barák L. Monitoring of early and advanced glycation in relation to the occurrence of microvascular complications in children and adolescents with type 1 diabetes mellitus. *Physiol Res* 2009; **58**: 553-561 [PMID: 18657005 DOI: 10.33549/physiolres.931612]

19 **Donaghue KC**, Marcovecchio ML, Wadwa RP, Chew EY, Wong TY, Calliari LE, Zabeen B, Salem MA, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. *Pediatr Diabetes* 2018; **19** Suppl 27: 262-274 [PMID: 30079595 DOI: 10.1111/pedi.12742]

20 **Alman AC**, Talton JW, Wadwa RP, Urbina EM, Dolan LM, Daniels SR, Hamman RF, D'Agostino RB, Marcovina SM, Mayer-Davis EJ, Dabelea DM. Cardiovascular health in adolescents with type 1 diabetes: the SEARCH CVD study. *Pediatr Diabetes* 2014; **15**: 502-510 [PMID: 24450411 DOI: 10.1111/pedi.12120]

21 **Guilmin-Crépon S**, Carel JC, Schroedt J, Scornet E, Alberti C, Tubiana-Rufi N. How should we assess glycemic variability in type 1 diabetes? *Diabetes Technol Ther* 2018; **20**: 440-447 [PMID: 29923773 DOI: 10.1089/dia.2017.0404]

22 **Giannopoulou EZ**, Doundoulakis I, Antza C, Christoforidis A, Haidich AB, Kotsis V, Stabouli S. Subclinical arterial damage in children and adolescents with type 1 diabetes: A systematic review and meta-analysis. *Pediatr Diabetes* 2019; **20**: 668-677 [PMID: 31173658 DOI: 10.1111/pedi.12874]

23 **Carlsen S**, Skrivarhaug T, Thue G, Cooper JG, Gøransson L, Løvaas K, Sandberg S. Glycemic control and complications in patients with type 1 diabetes - a registry-based longitudinal study of adolescents and young adults. *Pediatr Diabetes* 2017; **18**: 188-195 [PMID: 26875589 DOI: 10.1111/pedi.12372]

24 **Kochummen E**, Umpaichitra V, Marwa A, Joshi K, Chin VL, Perez-Colon S. Assessment of microvascular function in children and adolescents with diabetes and obesity. *Int J Endocrinol Metab* 2019; **18**: e90094 [PMID: 32308696 DOI: 10.5812/ijem.90094]

25 **Franks PW**, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC. Childhood obesity, other cardiovascular risk factors, and premature death. *N Engl J Med* 2010; **362**: 485-493 [PMID: 20147714 DOI: 10.1056/NEJMoa0904130]

26 **Naylor LH**, Davis EA, Kalic RJ, Paramalingam N, Abraham MB, Jones TW, Green DJ. Exercise training improves vascular function in adolescents with type 2 diabetes. *Physiol Rep* 2016; **4**: e12713 [PMID: 26887327 DOI: 10.14814/phy2.12713]

27 **Tryggestad JB**, Willi SM. Complications and comorbidities of T2DM in adolescents: findings from the TODAY clinical trial. *J Diabetes Complications* 2015; **29**: 307-312 [PMID: 25468310 DOI: 10.1016/j.jdiacomp.2014.10.009]

28 **Amutha A**, Mohan V. Diabetes complications in childhood and adolescent onset type 2 diabetes-a review. *J Diabetes Complications* 2016; **30**: 951-957 [PMID: 26970673 DOI: 10.1016/j.jdiacomp.2016.02.009]

29 **Bjornstad P**, Nehus E, El Ghormli L, Bacha F, Libman IM, McKay S, Willi SM, Laffel L, Arslanian S, Nadeau KJ; TODAY Study Group. Insulin sensitivity and diabetic kidney disease in children and adolescents with type 2 diabetes: An observational analysis of data from the TODAY Clinical Trial. *Am J Kidney Dis* 2018; **71**: 65-74 [PMID: 29157731 DOI: 10.1053/j.ajkd.2017.07.015]

30 **Rosen GP**, Nguyen HT, Shaibi GQ. Metabolic syndrome in pediatric cancer survivors: a mechanistic review. *Pediatr Blood Cancer* 2013; **60**: 1922-1928 [PMID: 23913590 DOI: 10.1002/pbc.24703]

31 **Rajendran R**, Abu E, Fadl A, Byrne CD. Late effects of childhood cancer treatment: severe hypertriglyceridaemia, central obesity, non alcoholic fatty liver disease and diabetes as complications of childhood total body irradiation. *Diabet Med* 2013; **30**: e239-e242 [PMID: 23692373 DOI: 10.1111/dme.12234]

32 **Baker KS**, Chow E, Steinberger J. Metabolic syndrome and cardiovascular risk in survivors after hematopoietic cell transplantation. *Bone Marrow Transplant* 2012; **47**: 619-625 [PMID: 21643022 DOI: 10.1038/bmt.2011.118]

33 **Friedman DN**, Hilden P, Moskowitz CS, Suzuki M, Boulad F, Kernan NA, Wolden SL, Oeffinger KC, Sklar CA. Cardiovascular risk factors in survivors of childhood hematopoietic cell transplantation treated with total body irradiation: A longitudinal analysis. *Biol Blood Marrow Transplant* 2017; **23**: 475-482 [PMID: 28040534 DOI: 10.1016/j.bbmt.2016.12.623]

34 **Leisenring WM**, Mertens AC, Armstrong GT, Stovall MA, Neglia JP, Lanctot JQ, Boice JD Jr,

Whitton JA, Yasui Y. Pediatric cancer survivorship research: experience of the Childhood Cancer Survivor Study. *J Clin Oncol* 2009; **27**: 2319-2327 [PMID: 19364957 DOI: 10.1200/JCO.2008.21.1813]

35 **Reinehr T**. Inflammatory markers in children and adolescents with type 2 diabetes mellitus. *Clin Chim Acta* 2019; **496**: 100-107 [PMID: 31276632 DOI: 10.1016/j.cca.2019.07.006]

36 **Babar G**, Clements M, Dai H, Raghuveer G. Assessment of biomarkers of inflammation and premature atherosclerosis in adolescents with type-1 diabetes mellitus. *J Pediatr Endocrinol Metab* 2019; **32**: 109-113 [PMID: 30710485 DOI: 10.1515/jpem-2018-0192]

37 **Anderson TJ**. Assessment and treatment of endothelial dysfunction in humans. *J Am Coll Cardiol* 1999; **34**: 631-638 [PMID: 10483941 DOI: 10.1016/S0735-1097(99)00259-4]

38 **Selamet Tierney ES**, Newburger JW, Gauvreau K, Geva J, Coogan E, Colan SD, de Ferranti SD. Endothelial pulse amplitude testing: feasibility and reproducibility in adolescents. *J Pediatr* 2009; **154**: 901-905 [PMID: 19217124 DOI: 10.1016/j.jpeds.2008.12.028]

39 **Lerman A**, Zeiher AM. Endothelial function: cardiac events. *Circulation* 2005; **111**: 363-368 [PMID: 15668353 DOI: 10.1161/01.CIR.0000153339.27064.14]

40 **Bonetti PO**, Pumper GM, Higano ST, Holmes DR Jr, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol* 2004; **44**: 2137-2141 [PMID: 15582310 DOI: 10.1016/j.jacc.2004.08.062]

41 **Celermajer DS**, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992; **340**: 1111-1115 [PMID: 1359209 DOI: 10.1016/0140-6736(92)93147-F]

42 **Bruyndonckx L**, Radtke T, Eser P, Vrints CJ, Ramet J, Wilhelm M, Conraads VM. Methodological considerations and practical recommendations for the application of peripheral arterial tonometry in children and adolescents. *Int J Cardiol* 2013; **168**: 3183-3190 [PMID: 23972967 DOI: 10.1016/j.ijcard.2013.07.236]

43 **Axtell AL**, Gomari FA, Cooke JP. Assessing endothelial vasodilator function with the Endo-PAT 2000. *J Vis Exp* 2010; **44**: 2167 [PMID: 20972417 DOI: 10.3791/2167]

44 **Bhangoo A**, Sinha S, Rosenbaum M, Shelov S, Ten S. Endothelial function as measured by peripheral arterial tonometry increases during pubertal advancement. *Horm Res Paediatr* 2011; **76**: 226-233 [PMID: 21778688 DOI: 10.1159/000328455]

45 **Radtke T**, Khattab K, Eser P, Kriemler S, Saner H, Wilhelm M. Puberty and microvascular function in healthy children and adolescents. *J Pediatr* 2012; **161**: 887-891 [PMID: 22632874 DOI: 10.1016/j.jpeds.2012.04.033]

46 **Chen Y**, Osika W, Dangardt F, Friberg P. Impact of psychological health on peripheral endothelial function and the HPA-axis activity in healthy adolescents. *Atherosclerosis* 2017; **261**: 131-137 [PMID: 28298251 DOI: 10.1016/j.atherosclerosis.2017.03.012]

47 **Olive LS**, Abhayaratna WP, Byrne D, Richardson A, Telford RD. Do self-reported stress and depressive symptoms effect endothelial function in healthy youth? *PLoS One* 2018; **13**: e0196137 [PMID: 29684063 DOI: 10.1371/journal.pone.0196137]

48 **Haller MJ**, Stein J, Shuster J, Theriaque D, Silverstein J, Schatz DA, Earing MG, Lerman A, Mahmud FH. Peripheral artery tonometry demonstrates altered endothelial function in children with type 1 diabetes. *Pediatr Diabetes* 2007; **8**: 193-198 [PMID: 17659060 DOI: 10.1111/j.1399-5448.2007.00246.x]

49 **Mahmud FH**, Van Uum S, Kanji N, Thiessen-Philbrook H, Clarson CL. Impaired endothelial function in adolescents with type 1 diabetes mellitus. *J Pediatr* 2008; **152**: 557-562 [PMID: 18346515 DOI: 10.1016/j.jpeds.2007.08.044]

50 **Pareyn A**, Allegaert K, Asscherickx W, Peirsman E, Verhamme P, Casteels K. Impaired endothelial function in female adolescents with type 1 diabetes measured by peripheral artery tonometry. *Eur J Pediatr* 2013; **172**: 1017-1022 [PMID: 23525544 DOI: 10.1007/s00431-013-1988-5]

51 **Mahmud FH**, Earing MG, Lee RA, Lteif AN, Driscoll DJ, Lerman A. Altered endothelial function in asymptomatic male adolescents with type 1 diabetes. *Congenit Heart Dis* 2006; **1**: 98-103 [PMID: 18377552 DOI: 10.1111/j.1747-0803.2006.00015.x]

52 **Scaramuzza AE**, Redaelli F, Giani E, Macedoni M, Giudici V, Gazzarri A, Bosetti A, De Angelis L, Zuccotti GV. Adolescents and young adults with type 1 diabetes display a high prevalence of endothelial dysfunction. *Acta Paediatr* 2015; **104**: 192-197 [PMID: 25424745 DOI: 10.1111/apa.12877]

53 **Deda L**, Yeshayahu Y, Sud S, Cuerden M, Cherney DZ, Sochett EB, Mahmud FH. Improvements in peripheral vascular function with vitamin D treatment in deficient adolescents with type 1 diabetes. *Pediatr Diabetes* 2018; **19**: 457-463 [PMID: 29063654 DOI: 10.1111/pedi.12595]

54 **Palombo C**, Kozakova M, Morizzo C, Gnesi L, Barsotti MC, Spontoni P, Massart F, Salvi P, Balbarini A, Saggese G, Di Stefano R, Federico G. Circulating endothelial progenitor cells and large artery structure and function in young subjects with uncomplicated type 1 diabetes. *Cardiovasc Diabetol* 2011; **10**: 88 [PMID: 21981808 DOI: 10.1186/1475-2840-10-88]

55 **Heier M**, Espeland CN, Brunborg C, Seljeflot I, Margeirsdottir HD, Hanssen KF, Fugelseth D, Dahl-Jørgensen K. Preserved endothelial function in young adults with type 1 diabetes. *PLoS One* 2018; **13**: e0206523 [PMID: 30359432 DOI: 10.1371/journal.pone.0206523]

56 **Chiesa ST**, Marcovecchio ML, Benitez-Aguirre P, Cameron FJ, Craig ME, Couper JJ, Davis EA,

Dalton RN, Daneman D, Donaghue KC, Jones TW, Mahmud FH, Marshall SM, Neil HAW, Dunger DB, Deanfield JE; Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT) Study Group. Vascular effects of ace (angiotensin-converting enzyme) inhibitors and statins in adolescents with type 1 diabetes. *Hypertension* 2020; **76**: 1734-1743 [PMID: 33100044 DOI: 10.1161/HYPERTENSIONAHA.120.15721]

57 **Scaramuzza A**, Giani E, Redaelli F, Ungheri S, Macedoni M, Giudici V, Bosetti A, Ferrari M, Zuccotti GV. Alpha-Lipoic Acid and antioxidant diet help to improve endothelial dysfunction in adolescents with type 1 diabetes: A Pilot Trial. *J Diabetes Res* 2015; **2015**: 474561 [PMID: 26171398 DOI: 10.1155/2015/474561]

58 **Donghui T**, Shuang B, Xulong L, Meng Y, Yujing G, Yujie H, Juan L, Dongsheng Y. Improvement of microvascular endothelial dysfunction induced by exercise and diet is associated with microRNA-126 in obese adolescents. *Microvasc Res* 2019; **123**: 86-91 [PMID: 30472037 DOI: 10.1016/j.mvr.2018.10.009]

59 **Pareyn A**, Allegaert K, Verhamme P, Vinckx J, Casteels K. Impaired endothelial function in adolescents with overweight or obesity measured by peripheral artery tonometry. *Pediatr Diabetes* 2015; **16**: 98-103 [PMID: 24698176 DOI: 10.1111/pedi.12139]

60 **Agarwal C**, Cohen HW, Muzumdar RH, Heptulla RA, Renukuntla VS, Crandall J. Obesity, hyperglycemia and endothelial function in inner city Bronx adolescents: a cross-sectional study. *Int J Pediatr Endocrinol* 2013; **2013**: 18 [PMID: 24164965 DOI: 10.1186/1687-9856-2013-18]

61 **Mahmud FH**, Hill DJ, Cuerden MS, Clarson CL. Impaired vascular function in obese adolescents with insulin resistance. *J Pediatr* 2009; **155**: 678-682 [PMID: 19595374 DOI: 10.1016/j.jpeds.2009.04.060]

62 **Tomsa A**, Klinepeter Bartz S, Krishnamurthy R, Bacha F. Endothelial function in youth: A Biomarker modulated by adiposity-related insulin resistance. *J Pediatr* 2016; **178**: 171-177 [PMID: 27546204 DOI: 10.1016/j.jpeds.2016.07.025]

63 **Del Ry S**, Cabiati M, Bianchi V, Caponi L, Maltinti M, Caselli C, Kozakova M, Palombo C, Morizzo C, Marchetti S, Randazzo E, Clerico A, Federico G. C-type natriuretic peptide is closely associated to obesity in Caucasian adolescents. *Clin Chim Acta* 2016; **460**: 172-177 [PMID: 27376982 DOI: 10.1016/j.cca.2016.06.045]

64 **Del Ry S**, Cabiati M, Bianchi V, Randazzo E, Peroni D, Clerico A, Federico G. C-type natriuretic peptide plasma levels and whole blood mRNA expression show different trends in adolescents with different degree of endothelial dysfunction. *Peptides* 2020; **124**: 170218 [PMID: 31794787 DOI: 10.1016/j.peptides.2019.170218]

65 **Singh R**, Verma A, Aljabari S, Vasylyeva TL. Urinary biomarkers as indicator of chronic inflammation and endothelial dysfunction in obese adolescents. *BMC Obes* 2017; **4**: 11 [PMID: 28344817 DOI: 10.1186/s40608-017-0148-2]

66 **Czippelova B**, Turianikova Z, Krohova J, Wiszt R, Lazarova Z, Pozorciakova K, Ciljakova M, Javorka M. Arterial stiffness and endothelial function in young obese patients - Vascular resistance matters. *J Atheroscler Thromb* 2019; **26**: 1015-1025 [PMID: 30930343 DOI: 10.5551/jat.47530]

67 **Bruyndonckx L**, Hoymans VY, Frederix G, De Guchtenaere A, Franckx H, Vissers DK, Vrints CJ, Ramet J, Conraads VM. Endothelial progenitor cells and endothelial microparticles are independent predictors of endothelial function. *J Pediatr* 2014; **165**: 300-305 [PMID: 24840759 DOI: 10.1016/j.jpeds.2014.04.015]

68 **Tryggestad JB**, Thompson DM, Copeland KC, Short KR. Obese children have higher arterial elasticity without a difference in endothelial function: the role of body composition. *Obesity (Silver Spring)* 2012; **20**: 165-171 [PMID: 21996664 DOI: 10.1038/oby.2011.309]

69 **Fusco E**, Pesce M, Bianchi V, Randazzo E, Del Ry S, Peroni D, Rossi M, Federico G. Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique. *Nutr Metab Cardiovasc Dis* 2020; **30**: 306-312 [PMID: 31653517 DOI: 10.1016/j.numecd.2019.09.007]

70 **Hudgins LC**, Annavajjhala V, Kovanlikaya A, Frank MD, Solomon A, Parker TS, Cooper RS. Non-invasive assessment of endothelial function in children with obesity and lipid disorders. *Cardiol Young* 2016; **26**: 532-538 [PMID: 25939357 DOI: 10.1017/S1047951115000657]

71 **Lowenstein L**, Damti A, Pillar G, Shott S, Blumenfeld Z. Evaluation of endothelial function in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* 2007; **134**: 208-212 [PMID: 17374431 DOI: 10.1016/j.ejogrb.2007.02.011]

72 **Orio F Jr**, Palomba S, Cascella T, De Simone B, Manguso F, Savastano S, Russo T, Tolino A, Zullo F, Lombardi G, Azziz R, Colao A. Improvement in endothelial structure and function after metformin treatment in young normal-weight women with polycystic ovary syndrome: results of a 6-month study. *J Clin Endocrinol Metab* 2005; **90**: 6072-6076 [PMID: 16118336 DOI: 10.1210/jc.2005-0965]

73 **Diamanti-Kandarakis E**, Alexandraki K, Protogerou A, Piperi C, Papamichael C, Aessopos A, Lekakis J, Mavrikakis M. Metformin administration improves endothelial function in women with polycystic ovary syndrome. *Eur J Endocrinol* 2005; **152**: 749-756 [PMID: 15879361 DOI: 10.1530/eje.1.01910]

74 **Bacha F**, Tomsa A, Bartz SK, Barlow SE, Chu ZD, Krishnamurthy R, Smith EO. Nonalcoholic fatty liver disease in hispanic youth with dysglycemia: Risk for subclinical atherosclerosis? *J Endocr Soc* 2017; **1**: 1029-1040 [PMID: 29264555 DOI: 10.1210/js.2017-00257]

75 **Kheirandish-Gozal L**, Etzioni T, Bhattacharjee R, Tan HL, Samiei A, Molero Ramirez H, Abu Eta B, Pillar G. Obstructive sleep apnea in children is associated with severity-dependent deterioration in overnight endothelial function. *Sleep Med* 2013; **14**: 526-531 [PMID: 23643649 DOI:

10.1016/j.sleep.2013.02.010]

76 **Chen Y**, Osika W, Dangardt F, Gan LM, Strandvik B, Friberg P. High levels of soluble intercellular adhesion molecule-1, insulin resistance and saturated fatty acids are associated with endothelial dysfunction in healthy adolescents. *Atherosclerosis* 2010; **211**: 638-642 [PMID: 20362293 DOI: 10.1016/j.atherosclerosis.2010.03.013]

77 **Blair CK**, Kelly AS, Steinberger J, Eberly LE, Napurski C, Robien K, Neglia JP, Mulrooney DA, Ross JA. Feasibility and preliminary efficacy of the effects of flavanoid-rich purple grape juice on the vascular health of childhood cancer survivors: a randomized, controlled crossover trial. *Pediatr Blood Cancer* 2014; **61**: 2290-2296 [PMID: 25175762 DOI: 10.1002/pbc.25202]

78 **Ruble K**, Davis CL, Han HR. Endothelial health in childhood acute lymphoid leukemia survivors: pilot evaluation with peripheral artery tonometry. *J Pediatr Hematol Oncol* 2015; **37**: 117-120 [PMID: 24577544 DOI: 10.1097/MPH.0000000000000122]

79 **Dengel DR**, Ness KK, Glasser SP, Williamson EB, Baker KS, Gurney JG. Endothelial function in young adult survivors of childhood acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2008; **30**: 20-25 [PMID: 18176175 DOI: 10.1097/MPH.0b013e318159a593]

80 **Jenei Z**, Bárdi E, Magyar MT, Horváth A, Paragh G, Kiss C. Anthracycline causes impaired vascular endothelial function and aortic stiffness in long term survivors of childhood cancer. *Pathol Oncol Res* 2013; **19**: 375-383 [PMID: 23242567 DOI: 10.1007/s12253-012-9589-6]

81 **Zelcer S**, Chen B, Mangel J, Vujovic O, Thiessen-Philbrook HR, Reider M, Mahmud FH. Impaired vascular function in asymptomatic young adult survivors of Hodgkin Lymphoma following mediastinal radiation. *J Cancer Surviv* 2010; **4**: 218-224 [PMID: 20652436 DOI: 10.1007/s11764-010-0138-6]

82 **Vatanen A**, Sarkola T, Ojala TH, Turanlahti M, Jahnukainen T, Saarinen-Pihkala UM, Jahnukainen K. Radiotherapy-related arterial intima thickening and plaque formation in childhood cancer survivors detected with very-high resolution ultrasound during young adulthood. *Pediatr Blood Cancer* 2015; **62**: 2000-2006 [PMID: 26052933 DOI: 10.1002/pbc.25616]

83 **Chatrath R**, Ronningen KL, LaBreche P, Severson SR, Jayachandran M, Bracamonte MP, Miller VM. Effect of puberty on coronary arteries from female pigs. *J Appl Physiol (1985)* 2003; **95**: 1672-1680 [PMID: 12794029 DOI: 10.1152/japplphysiol.00099.2003]

84 **Chatrath R**, Ronningen KL, Severson SR, LaBreche P, Jayachandran M, Bracamonte MP, Miller VM. Endothelium-dependent responses in coronary arteries are changed with puberty in male pigs. *Am J Physiol Heart Circ Physiol* 2003; **285**: H1168-H1176 [PMID: 12738626 DOI: 10.1152/ajpheart.00029.2003]

85 **Duke PM**, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980; **66**: 918-920 [PMID: 7454482]

86 **Higashi Y**, Sukhanov S, Anwar A, Shai SY, Delafontaine P. IGF-1, oxidative stress and atheroprotection. *Trends Endocrinol Metab* 2010; **21**: 245-254 [PMID: 20071192 DOI: 10.1016/j.tem.2009.12.005]

87 **Suzuki K**, Yanagi K, Shimizu M, Wakamatsu S, Niitani T, Hosonuma S, Sagara M, Aso Y. Effect of growth hormone replacement therapy on plasma diacron-reactive oxygen metabolites and endothelial function in Japanese patients: The GREAT clinical study. *Endocr J* 2018; **65**: 101-111 [PMID: 29070768 DOI: 10.1507/endocrj.EJ17-0330]

88 **Caicedo D**, Díaz O, Devesa P, Devesa J. Growth Hormone (GH) and Cardiovascular System. *Int J Mol Sci* 2018; **19** [PMID: 29346331 DOI: 10.3390/ijms19010290]

89 **Conti E**, Musumeci MB, De Giusti M, Dito E, Mastromarino V, Autore C, Volpe M. IGF-1 and atherothrombosis: relevance to pathophysiology and therapy. *Clin Sci (Lond)* 2011; **120**: 377-402 [PMID: 21244364 DOI: 10.1042/CS20100400]

90 **Lanes R**, Soros A, Flores K, Gunczler P, Carrillo E, Bandel J. Endothelial function, carotid artery intima-media thickness, epicardial adipose tissue, and left ventricular mass and function in growth hormone-deficient adolescents: apparent effects of growth hormone treatment on these parameters. *J Clin Endocrinol Metab* 2005; **90**: 3978-3982 [PMID: 15870123 DOI: 10.1210/jc.2005-0091]

91 **Colao A**, Spinelli L, Cuocolo A, Spiezia S, Pivonello R, di Somma C, Bonaduce D, Salvatore M, Lombardi G. Cardiovascular consequences of early-onset growth hormone excess. *J Clin Endocrinol Metab* 2002; **87**: 3097-3104 [PMID: 12107207 DOI: 10.1210/jcem.87.7.8573]

92 **Colao A**, Ferone D, Marzullo P, Lombardi G. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. *Endocr Rev* 2004; **25**: 102-152 [PMID: 14769829 DOI: 10.1210/er.2002-0022]

93 **Kul S**, Caklili OT, Tutuncu Y, Ozcan FB, Aksu F, Baycan OF, Atici A, Bilgili UZ, Takir M, Caliskan M. Endothelial dysfunction in patients with acromegaly and It's association with Endocan. *Growth Horm IGF Res* 2021; **56**: 101362 [PMID: 33221710 DOI: 10.1016/j.ghir.2020.101362]

94 **O'Gorman CS**, Syme C, Bradley T, Hamilton J, Mahmud FH. Impaired endothelial function in pediatric patients with Turner syndrome and healthy controls: a case-control study. *Int J Pediatr Endocrinol* 2012; **2012**: 5 [PMID: 22472028 DOI: 10.1186/1687-9856-2012-5]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 827-838

DOI: 10.4239/wjd.v12.i6.827

ISSN 1948-9358 (online)

MINIREVIEWS

Diabetes and peripheral artery disease: A review

David Olubukunmi Soyoye, Olugbenga Olusola Abiodun, Rosemary Temidayo Ikem, Babatope Ayodeji Kolawole, Anthony Olubunmi Akintomide

ORCID number: David Olubukunmi Soyoye 0000-0002-9659-2658; Olugbenga Olusola Abiodun 0000-0001-8625-4834; Rosemary Temidayo Ikem 0000-0003-1099-5438; Babatope Ayodeji Kolawole 0000-0002-8242-3968; Anthony Olubunmi Akintomide 0000-0003-3048-7027.

Author contributions: All the authors contributed to the initial writing and subsequent reviews of this manuscript.

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

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

David Olubukunmi Soyoye, Rosemary Temidayo Ikem, Babatope Ayodeji Kolawole, Anthony Olubunmi Akintomide, Department of Medicine, Obafemi Awolowo University, Ile-Ife 220282, Osun State, Nigeria

David Olubukunmi Soyoye, Rosemary Temidayo Ikem, Babatope Ayodeji Kolawole, Anthony Olubunmi Akintomide, Department of Medicine, Obafemi Awolowo University Teaching Hospital, Ile-Ife 220282, Osun State, Nigeria

Olugbenga Olusola Abiodun, Department of Medicine, Federal Medical Centre, Jabi 900211, Abuja, Nigeria

Corresponding author: David Olubukunmi Soyoye, FMCP, FACE, FACP, MBChB, Consultant Physician-Scientist, Senior Lecturer, Department of Medicine, Obafemi Awolowo University, Ife-Ibadan Expressway, Ile-Ife 220282, Osun State, Nigeria. bksoyoye@yahoo.com

Abstract

Peripheral arterial disease (PAD) refers to partial or complete occlusion of the peripheral vessels of the upper and lower limbs. It usually occurs as part of systemic atherosclerosis in the coronary and cerebral arteries. The prevalence of PAD is expected to continue to increase in the foreseeable future owing to the rise in the occurrence of its major risk factors. Nonhealing ulcers, limb amputation and physical disability are some of its major complications. Diabetes mellitus (DM) remains a major risk for PAD, with DM patients having more than two-fold increased prevalence of PAD compared with the general population. The clinical presentation in people with DM also differs slightly from that in the general population. In addition, PAD in DM may lead to diabetic foot ulcers (DFUs), which precipitate hyperglycaemic emergencies and result in increased hospital admissions, reduced quality of life, and mortality. Despite the epidemiological and clinical importance of PAD, it remains largely under diagnosed and hence undertreated, possibly because it is largely asymptomatic. Emphasis has been placed on neuropathy as a cause of DFUs, however PAD is equally important. This review examines the epidemiology, pathophysiology and diagnosis of lower limb PAD in people with diabetes and relates these to the general population. It also highlights recent innovations in the management of PAD.

Key Words: Diabetes; Peripheral arterial disease; Diabetic foot ulcers; Lower limb complications

Country/Territory of origin: Nigeria

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

Received: February 13, 2021
Peer-review started: February 13, 2021
First decision: March 16, 2021
Revised: April 22, 2021
Accepted: May 7, 2021
Article in press: May 7, 2021
Published online: June 15, 2021

P-Reviewer: Yuan B
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Ma YJ

Core Tip: Peripheral arterial disease (PAD) is a major cause of nonhealing ulcers, lower limb amputation and mortality, especially in people with diabetes. The ominous association between PAD and diabetic foot disease is largely under-reported. Hence, it is under diagnosed and undertreated. This article reviews the impact of PAD in diabetes, its traditional and non-traditional risk factors, and pathophysiology, and examines some recent innovations in its management.

Citation: Soyoye DO, Abiodun OO, Ikem RT, Kolawole BA, Akintomide AO. Diabetes and peripheral artery disease: A review. *World J Diabetes* 2021; 12(6): 827-838
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/827.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.827

INTRODUCTION

Diabetes mellitus (DM) continues to assume pandemic proportions, affecting people across various socioeconomic groups in developed and developing nations. Globally, close to a half billion people are living with diabetes and it is expected to increase by more than 50% in the next 25 years[1]. The myriad of chronic complications attributable to the disease results in enormous physical, mental, and economic burdens. The complications are mainly vascular and lead to diabetes-specific microvascular sequelae in the retina, nerves and the glomerulus. Others are atherosclerotic macrovascular pathology in the brain, heart and lower limbs[2].

Lower extremity complications are common, showing a rising trend in many regions of the world and affecting about 131 million people worldwide, with an estimated global prevalence of 1.8%[3]. They significantly impact morbidity and mortality in people with DM, sometimes leading to leg ulcers and amputations, which are generally characterized by physical disability, reduced productivity and emotional disturbances. Although much emphasis has been laid on neuropathy as a cause, an equally important contributor to the occurrence of leg ulcers and amputations is peripheral arterial disease (PAD)[2,4-6]. Consequently, PAD is under-diagnosed and hence, may be undertreated.

PAD denotes a complete or partial occlusion of one or more of the noncardiac, non-intracranial, peripheral arteries of the upper and lower limbs, which may lead to reduced blood flow or tissue loss[7]. It usually results from atherosclerosis of the vessel wall, but may also arise as a result of embolism, thrombosis, fibromuscular dysplasia, or vasculitis[7]. Atherosclerotic PAD may be a pointer to systemic atherosclerosis in non-peripheral intra-cerebral and coronary arteries. In DM, the arteries of the lower limbs are the ones that are mostly involved; and most often the distal arteries, especially the dorsalis pedis artery[8]. This review discusses the pathophysiology of atherosclerotic PAD of the lower limbs, its epidemiology in DM, and its treatment. It also highlights recent advances in its management.

EPIDEMIOLOGY OF PAD IN DIABETES

The prevalence of PAD depends on the diagnostic measurement employed, cut-off values of the test, the limb assessed and the population studied[9]. It has been assessed using the presence of intermittent claudication (IC), palpation of the vessels of the lower limbs, and measurement of the ankle-brachial index (ABI). Prevalence generally increases with advancing age, irrespective of the measurement utilized. IC, the main symptom attributable to PAD, occurred in about 1.5% of the cohorts in the Framingham Heart Study. In all age groups, the rate in men was double that in women[10]. Also, in the Rotterdam study involving the elderly population, IC was reported by 1.6% of the participants, but the prevalence of PAD defined by an ABI < 0.9 in either leg was 19.1% in the same cohort[11]. The prevalence in men was higher in both studies. The rates of PAD using IC is generally lower compared with those obtained using ABI[11-13].

In community studies, the prevalence of PAD using ABI differs with the population, cut-off value, ankle vessel and the leg used, with values ranging from 4.3% to 9.0% in the general population[14,15]. In a systemic review assessing community-based studies of the global prevalence of PAD (using ABI ≤ 0.9) and its risk factors, prevalence differed based on the region studied and sex. It was higher among men in high-income countries, and in women in low- and middle-income countries[16]. Certain factors affect the accurate assessment of PAD in people with diabetes. PAD is often asymptomatic; the presence of peripheral neuropathy, which is a common complication of DM. may distort pain perception, and the presence of IC and absence of peripheral pulses are inadequate diagnostic indicators[8].

In hospital-based studies, PAD is two- to seven-fold more prevalent in people with diabetes than it is in those without it, with rates between 9% and 55% in people with diabetes[5,17-19]. In a national survey involving about 3000 adult Americans 40 years of age and above, PAD was two times more prevalent in people with diabetes compared with the general population[20]. Also, a systematic review of studies comparing PAD in diabetics and nondiabetics reported that PAD ranged between 20% and 50% in those with diabetes, compared with 10% and 26% in those without diabetes[21]. Also, as seen in the general population, the prevalence of PAD differed depending on the diagnostic method used (IC, palpation of vessels or ABI)[19].

Lower limb amputation resulting from foot ulcers is a major cause of disability, especially in diabetic patients. Patients with foot ulcers are more likely to present with PAD than those without ulcers, with the attendant increased mortality and lower limb amputations in those patient cohorts[22-25].

RISK FACTORS FOR PAD

The major risk factors for PAD such as DM, hypertension, smoking and hyperlipidaemia also contribute to coronary heart disease (CHD) and cerebrovascular disease (CVD). However, the influence exerted by those risks on vascular diseases is different [26-28]. In a recent systemic review that assessed community-based studies for global prevalence and risk factors of PAD, DM ranked next to smoking among the major risks and hypertension and hypercholesterolaemia followed[16,29]. In the National Health and Nutrition Examination Survey, cigarette smoking and DM were also the most significant risk factors for PAD, with a odds ratios of 4.5 and 2.7, respectively[14].

In other community-based studies, diabetes also ranked high as a risk factor for the occurrence and progression of PAD along with other traditional risks such as age, smoking, hypertension, hypercholesterolaemia and low kidney function)[11-13,30,31]. It hiked the rates of lower extremity amputation, hospital stay, and mortality[21,22,26]. While the major risk factors for PAD in people without DM remain significant even with it, other associations have also been identified in DM. They include longer duration of DM, high glycated haemoglobin (HBA1c) level, abdominal obesity, male sex and neuropathy[18,19,22,32].

The traditional risk factors do not fully explain the development of atherosclerosis in the peripheral or other vascular beds. Inflammation, abnormalities in haemostasis and blood viscosity are known to contribute to the evolution and propagation of atherosclerosis, and their markers have been studied[33-35]. High-sensitivity C-reactive protein, hyperuricaemia, and hyperhomocysteinaemia are some of the non-traditional risk factors associated with PAD in the general population and in people with DM[18,19,36-38].

PATHOPHYSIOLOGY

The central pathophysiological theme of PAD in DM is the process of atherosclerosis. It begins with atherogenesis, and progresses to the eventual obstruction and reduction of blood flow. In what is known as subclinical atherosclerosis, the pathological changes may predate the diagnosis of impaired fasting glucose and DM[39]. The changes are the same as those observed in other vascular beds in patients with DM. Several pathogenetic mechanisms have been identified in the initiation of atherosclerosis, including endothelial dysfunction, inflammation, platelet aggregation and vascular smooth muscle cell (VSMC) dysfunction[40]. Figure 1 shows a schematic representation of these factors and how they lead to PAD.

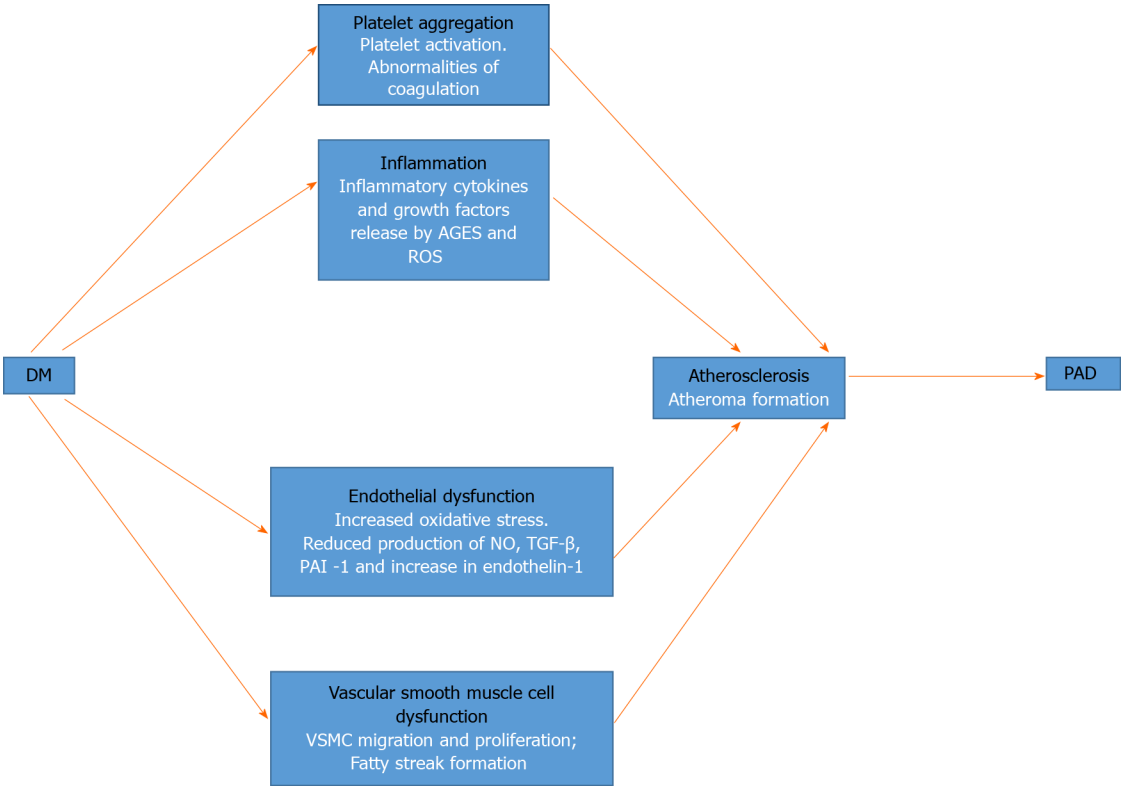

Figure 1 Schematic representation of the pathophysiology of peripheral arterial disease in diabetes mellitus. Pathogenetic processes and their mechanisms are shown in black and white type, respectively. AGEs: Advanced glycation end products; DM: Diabetes mellitus; NO: Nitric oxide; PAD: Peripheral arterial disease; PAI-1: Plasminogen activator inhibitor-1; ROS: Reactive oxygen species; TGF-β: Transforming growth factor-beta; VSMC: Vascular smooth muscle cell.

Dysfunction of the vascular endothelium is the hallmark of atherosclerosis in DM, and it arises from a variety of inter-related pathogenetic factors. First, chronic hyperglycaemia activates the dormant polyol pathway. That results in increased oxidative stress from reactive oxygen species, caused by the consumption of cofactor nicotinamide adenine dinucleotide phosphate and reduced glutathione[41,42]. Chronic hyperglycaemia also causes the production of advanced glycation end products, which results in the elaboration of inflammatory cytokines and growth factors that cause vascular injury[42]. In addition, hyperglycaemia induces the activation of protein kinase C, which has various effects on gene expression. Protein kinase C is responsible for the activation of the nuclear factor κB, a transcription factor that activates a variety of proinflammatory genes[42,43]. The resultant effect is a reduction in the production of nitric oxide (NO), which is a potent vasodilator; transforming growth factor (TGF)-β and plasminogen activator inhibitor (PAI)-1. The production of the vasoconstrictor endothelin-1 is increased. NO reduces inflammation by modulating leucocyte-vascular wall interaction and inhibiting VSMC migration and platelet activation[40]. Those abnormalities in the absence of NO, result in increased endothelial permeability, leucocyte chemotaxis, adhesion and migration into the intima, thus causing inflammation. There is also low-density lipoprotein (LDL) migration into the intima where it is oxidized within monocytes to form foam cells, which are the earliest precursor of atheroma formation.

Endothelial injury and hyperglycaemia are activators of platelet adhesion, activation and aggregation. With hyperglycaemia, glucose uptake by platelets is left unchecked, resulting in platelet activation and increased oxidative stress through the release of reactive oxygen species[40]. Also, hyperglycaemia is associated with abnormalities of coagulation such as the decreased concentration of antithrombin and protein C, impaired fibrinolytic function and excess production of PAI-1[40]. Platelet activation and aggregation are therefore important elements in the development of atherosclerosis.

Hyperglycaemia is also associated with VSMC dysfunction through the effects of endothelial injury and intima inflammation. Proinflammatory mediators such as platelet-derived growth factors (PDGFs), vascular endothelial growth factors, and cytokines released in the inflammatory milieu of the intima result in VSMC migration

and proliferation. The combination of VSMC and endothelial foam cells subsequently results in the development of fatty streaks that become remodelled into an atheromatous plaque. The plaque is the result of collagen production and an extracellular matrix by VSMC through the mediating effects of PDGF and TGF-β[40,44]. The increasing size of the atheromatous plaque, which causes obstruction and reduction of blood flow, is the hallmark of atherosclerosis as seen in PAD and other vascular beds in DM patients.

DIAGNOSTIC EVALUATION

History and physical examination

History taking in all DM patients should entail asking for risk factors for PAD, such as hypertension, dyslipidaemia, cigarette smoking, obesity and the duration of DM. Patients who have been diabetic for more than 10 years are more prone to the risk of PAD[19,31,45]. Similarly, longer duration of, and exposure to higher levels of the other factors (hypertension, dyslipidaemia, smoking, obesity) potentiates the risk of PAD [31]. History taking should also focus on the presence of other macrovascular complications such as CVD and CHD because they are equivalents. Symptoms of PAD include IC in about 10% of patients; pain at rest, which is indicative of critical limb ischaemia, and about 50% of patients will be asymptomatic[46]. Examiners should search for differentials of PAD such as pseudo-claudication in spinal stenosis, peripheral neuropathy, nerve root compression, deep venous thrombosis, vasculitis and musculoskeletal causes such as arthritis[46]. Examination may reveal features of ischaemia such as dependent rubor, elevated pallor, and shiny and hairless skin. Also, peripheral pulses such as the femoral, popliteal, posterior tibial and dorsalis pedis arteries may be reduced. Some patients may present with trophic skin changes and gangrene.

ABI

The ABI is a sensitive and specific screening tool for PAD. It has a sensitivity of 90% and specificity of 98% in detecting PAD[47]. The European Society of Cardiology (ESC), and American Heart Association recommend the use of ABI to screen for PAD in all diabetics older than 50 years of age. Others include diabetics younger than 50 years of age with a DM duration of more than 10 years or with other risk factors for PAD such as smoking, hypertension, dyslipidaemia and PAD equivalents[45,48]. An ABI of < 0.9 is indicative of PAD, and is associated with a 2- to 4 -fold increase in mortality[45]. An ABI of > 1.3 is indicative of poorly compressible vessels resulting from vascular calcification, which is also associated with an increased risk of mortality and amputation (Table 1)[40].

Duplex ultrasound

Duplex ultrasound is a combination of conventional and doppler ultrasonography. It is indicated as a first-line imaging method to detect the site and extent of severity of vascular lesions[45].

Computed tomography and magnetic resonance angiography

Angiography is indicated in patients with planned revascularization to guide optimal revascularization strategies. Computed tomography angiography is non-invasive, widely available, and has a high resolution. The disadvantages include exposure to irradiation, use of iodinated contrast agents and contrast nephrotoxicity, particularly in patients with chronic kidney disease (CKD)[45]. Magnetic resonance angiography has the advantage of being acceptable in mild to moderate CKD, with higher soft-tissue resolution. It is limited by frequent motion artefacts, claustrophobia, severe CKD, and in patients with magnetic resonance imaging noncompliant pacemakers or implantable cardioverter defibrillators[45].

TREATMENT

The management of PAD in DM includes symptomatic control and reduction of the risk of cardiovascular (CV) events. Management includes CV risk factor treatment and lifestyle modifications such as regular physical exercise, promotion of a healthy diet, weight reduction and smoking cessation. If medical management fails because of disabling symptoms or in the presence of chronic life-threatening ischaemia, then

Table 1 Interpretation of ankle-brachial index

Ankle-brachial index	Interpretation
< 0.4	Severe obstruction
0.4-0.69	Moderate obstruction
0.7-0.90	Mild obstruction
0.91-1.30	Normal
> 1.30	Poorly compressible

revascularization is indicated.

Exercise

Regular physical activity improves claudication distance in PAD. It also improves quality of life and reduces the risk of CV disease, which often accompanies PAD[49,50]. Home-based walking exercise is recommended for a minimum of 30 min, at least 3 d of the week[49]. Randomized controlled trials of 493 patients with PAD showed that home-based walking exercise improved walking ability in patients and also improved 6-min walk more than supervised treadmill exercise[51].

Statins

High-intensity statin therapy is recommended for all patients with PAD[52,53]. Observational and randomized clinical studies have shown that statin therapy reduced all-cause mortality and CV events in patients with PAD. The goal is to reduce LDL cholesterol (LDL-C) to < 1.8 mmol/L (70 mg/dL) or to reduce it by ≥ 50% if baseline values are 1.8-3.5 mmol/L (70-135 mg/dL)[45]. In statin-benefit groups such as PAD, ezetimibe is a reasonable and beneficial addition if LDL-C remains > 1.8 mmol/L (70 mg/dL) with maximally tolerated statin therapy[45,54]. If LDL-C remains > 1.8 mmol/L (70 mg/dL) on statins and ezetimibe, the addition of evolocumab, a monoclonal antibody that inhibits proprotein convertase subtilisin/kexin type 9 is reasonable and beneficial to reduce CV events[45,55].

Antiplatelet therapy

Single antiplatelet therapy is indicated in all patients with symptomatic PAD and in those who have had revascularization[45]. Antiplatelet agents are effective in preventing limb-related and CV events[56]. A post hoc analysis of the CAPRIE (Clopidogrel *vs* Aspirin in Patients at Risk of Ischaemic Events) trial in 6452 patients with clinical lower extremity artery disease (LEAD) showed that at 3 years, clopidogrel was superior with significant reductions in CV mortality [hazard ratio (HR) 0.76 (95%CI: 0.64-0.91)] and major adverse cardiovascular events HR 0.78 (95%CI: 0.65-0.93)][57]. A similar benefit was seen in the subgroup of LEAD patients with DM[57]. In the randomized EUCLID (Effects of Ticagrelor and Clopidogrel in Patients with Peripheral Artery Disease) trial, ticagrelor did not show any difference compared to clopidogrel[58]. Clopidogrel is therefore the recommended antiplatelet drug in symptomatic PAD.

Vasodilators

Cilostazol, an oral phosphodiesterase type III inhibitor is useful in managing IC. It inhibits platelet aggregation and causes vasodilation. Randomized controlled trials have shown improved walking distance and quality of life with the use of cilostazol [59]. However, it has been suggested that improvement in walking distance is mild to moderate, with great variability.

Glycaemic control

There are no randomized controlled trials with arms comparing intensive or standard arm glucose lowering in those with DM and PAD. However, there is evidence that glucose control is associated with a reduction in microvascular and macrovascular complications. In type 1 DM, the DCCT (Diabetes Control and Complications Trial) showed a reduction in CV events in the intensive arm compared with the standard arm, both with long-term follow-up[60]. With long-term follow-up, intensive control showed a 57% reduction in nonfatal myocardial infarction (MI), stroke, CV death, as well as some reduction in all-cause mortality[61]. However, in type 2 DM, evidence of

the benefit of intensive lowering of glycaemia was not as compelling. There is a need, therefore, for a general goal of a glycated haemoglobin level of < 7%, while individualizing the goal of treatment for each patient's characteristics[62].

In the UKPDS (United Kingdom Prospective Diabetic Study), short-term follow-up did not show a significant benefit in the reduction of CV events of combined fatal and nonfatal MI, sudden death (P = 0.052) and stroke[63]. However, after 10 years of follow-up, patients in the intensive glycaemia control arm showed significant reductions in MI and all-cause mortality[63]. The short-term and long-term results of the UKPDS are contradicted by those of ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial, the ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation) trial and the VADT (Veterans Affairs Diabetic Trial[64]. In those trials, short-term follow-up of 3.5 to 5.6 years did not show any reduction in CV events in those in the intensive arm. Long-term follow-up in the ADVANCE trial showed no evidence of CV benefit or harm[65]. In the ACCORD trial, the glycaemic control comparison was stopped early because of increased mortality in the intensive (1.41% per year) compared with the standard (1.14% per year) treatment arms [HR 1.22 (95%CI: 1.01-1.46)]. But the long-term follow-up at 10 years in the VADT showed a reduction in CV events in the intensive arm[66, 67]. In those three trials, the patients had high CV risks, longer DM duration, and were relatively older than patients in UKPDS. Also, severe hypoglycaemia was more likely in the intensive arms, hence the importance of individualizing control for those groups of patients to reduce the high risk of CV events and mortality because of hypoglycaemia.

Blood pressure control

The ESC/European Society of Hypertension recommends that systolic blood pressure (SBP) be targeted to < 130 mmHg and that the diastolic blood pressure should < 80 mmHg and that the SBP should not be < 120 mmHg in patients with DM[68]. This is supported by evidence from the ONTARGET (Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial) and ACCORD trials showing an overall reduction in CV events with intensive SBP lowering to < 130 mmHg[69,70]. Diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and beta-blockers can all be used in PAD. However, the HOPE (Heart Outcomes Prevention) and ONTARGET studies have shown that ACEIs and ARBs reduce CV events in PAD and should therefore be considered in treating patients with PAD and hypertension with or without DM[69, 71]. Care should be taken with the use of beta-blockers in patients with chronic limb-threatening ischaemia[68].

Revascularization

Revascularization is indicated if claudication impairs quality of life after the failure of exercise therapy and pharmacotherapy in patients whose general condition allows invasive treatment. Strategies include endovascular therapy, open surgery, or a combination of the two. Endovascular therapy is generally recommended for short (< 25 cm) occlusive lesions, and in those with high surgical risk. It includes balloon dilation (angioplasty), stents, and atherectomy[72]. Open surgery is recommended for patients with long (≥ 25 cm) lesions who are young and fit[45].

RECENT INNOVATIONS IN THE MANAGEMENT OF PAD

Endovascular therapy has continued to evolve with the modification and development of new technologies, including drug-eluting stents, self-expanding stents, cutting balloons (CBs), and cryoplasty balloons. Other interventions are focal pressure balloons and drug-coated balloons (DCBs). These reduce post-treatment cell proliferation or restenosis, thereby improving patency, and new atherectomy systems, especially for calcified lesions[72-74]. CBs and cryoplasty balloons are modifications of standard percutaneous transluminal angioplasty (PTA). PTA is done by placing a wire within the artery beyond the target lesion and then expanding the inserted balloon with appropriate pressure. That leads to fracture of the lesion and stretching of the arterial wall[73]. Cryoplasty balloons induce an inflammatory response and dilate plaques by utilizing a combination of hypothermia and pressure. DCBs inhibit hyperplasia by including medication (usually paclitaxel) after performing a standard PTA[73].

Lithoplasty (Shockwave Medical) is an atherectomy device that combines a balloon angioplasty catheter with sound waves that break up calcifications that otherwise would not be broken with the use of DCBs and stents[74]. The Pantheris Lumivascular Atherectomy System (Avinger, Inc.) is a directional atherectomy system that includes optical coherence tomography. It utilizes light to provide three-dimensional visual guidance rather than two-dimensional X-ray images with fluoroscopy. It aids better navigation for removal of plaque, reduces damage to the artery and may reduce exposure to radiation from fluoroscopic imaging procedures[74].

CONCLUSION

DM is a major risk for PAD, resulting in increased morbidity and mortality. Morbidity is characterized by an increased risk of other cardiovascular complications, increased hospital admissions, disability from leg ulcers and amputation, reduced productivity and reduced quality of life.

Early detection of PAD in diabetic patients at risk is imperative to reduce morbidity and mortality. At-risk diabetics include older patients, those with a DM duration longer than 10 years, high HBA1c, obesity and neuropathy. The ABI is a highly sensitive and specific simple tool to screen for PAD in DM. It is also valuable as a follow-up tool, and also for stratifying CV risk[45].

Prevention of CV events and symptom control in symptomatic patients are the paramount pillars of the treatment of PAD in DM. They should include treatment of CV risk factors, and treatment of PAD, including pharmacological and nonpharmacological interventions and revascularization if medical treatment fails.

Open surgery used to be the mainstay of revascularization, endovascular therapy has however evolved recently to improve outcomes, with the development of new innovations, such as the DES, CBs, self-expanding stents, and cryoplasty balloons. More studies are needed to evaluate quality of life and wound healing using these newer endovascular modalities and to compare surgical and endovascular revascularization in symptomatic patients[45].

REFERENCES

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

2 Standards of Medical Care in Diabetes-2016: Summary of Revisions. *Diabetes Care* 2016; **39** Suppl 1: S4-S5 [PMID: 26696680 DOI: 10.2337/dc16-S003]

3 **Zhang Y**, Lazzarini PA, McPhail SM, van Netten JJ, Armstrong DG, Pacella RE. Global Disability Burdens of Diabetes-Related Lower-Extremity Complications in 1990 and 2016. *Diabetes Care* 2020; **43**: 964-974 [PMID: 32139380 DOI: 10.2337/dc19-1614]

4 **Akanji AO**, Adetuyidi A. The pattern of presentation of foot lesions in Nigerian diabetic patients. *West Afr J Med* 1990; **9**: 1-5 [PMID: 2271417]

5 **Ikem R**, Ikem I, Adebayo O, Soyoye D. An assessment of peripheral vascular disease in patients with diabetic foot ulcer. *Foot (Edinb)* 2010; **20**: 114-117 [PMID: 20951569 DOI: 10.1016/j.foot.2010.09.002]

6 **Peters EJ**, Armstrong DG, Lavery LA. Risk factors for recurrent diabetic foot ulcers: site matters. *Diabetes Care* 2007; **30**: 2077-2079 [PMID: 17507693 DOI: 10.2337/dc07-0445]

7 **Kullo IJ**, Rooke TW. CLINICAL PRACTICE. Peripheral Artery Disease. *N Engl J Med* 2016; **374**: 861-871 [PMID: 26962905 DOI: 10.1056/NEJMcp1507631]

8 **Marso SP**, Hiatt WR. Peripheral arterial disease in patients with diabetes. *J Am Coll Cardiol* 2006; **47**: 921-929 [PMID: 16516072 DOI: 10.1016/j.jacc.2005.09.065]

9 **Hiatt WR**, Hoag S, Hamman RF. Effect of diagnostic criteria on the prevalence of peripheral arterial disease. The San Luis Valley Diabetes Study. *Circulation* 1995; **91**: 1472-1479 [PMID: 7867189 DOI: 10.1161/01.cir.91.5.1472]

10 **Murabito JM**, D'Agostino RB, Silbershatz H, Wilson WF. Intermittent claudication. A risk profile from The Framingham Heart Study. *Circulation* 1997; **96**: 44-49 [PMID: 9236415 DOI: 10.1161/01.cir.96.1.44]

11 **Meijer WT**, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE. Peripheral arterial disease in the elderly: The Rotterdam Study. *Arterioscler Thromb Vasc Biol* 1998; **18**: 185-192 [PMID: 9484982 DOI: 10.1161/01.atv.18.2.185]

12 **Bennett PC**, Lip GY, Silverman S, Blann AD, Gill PS. The contribution of cardiovascular risk factors

to peripheral arterial disease in South Asians and Blacks: a sub-study to the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) study. *QJM* 2010; **103**: 661-669 [PMID: 20576717 DOI: 10.1093/qjmed/hcq102]

13 **Ramos R**, Quesada M, Solanas P, Subirana I, Sala J, Vila J, Masiá R, Cerezo C, Elosua R, Grau M, Cordón F, Juvinyà D, Fitó M, Isabel Covas M, Clarà A, Angel Muñoz M, Marrugat J; REGICOR Investigators. Prevalence of symptomatic and asymptomatic peripheral arterial disease and the value of the ankle-brachial index to stratify cardiovascular risk. *Eur J Vasc Endovasc Surg* 2009; **38**: 305-311 [PMID: 19515589 DOI: 10.1016/j.ejvs.2009.04.013]

14 **Selvin E**, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National Health and Nutrition Examination Survey, 1999-2000. *Circulation* 2004; **110**: 738-743 [PMID: 15262830 DOI: 10.1161/01.CIR.0000137913.26087.F0]

15 **Fowkes FG**, Housley E, Cawood EH, Macintyre CC, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol* 1991; **20**: 384-392 [PMID: 1917239 DOI: 10.1093/ije/20.2.384]

16 **Fowkes FG**, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, Norman PE, Sampson UK, Williams LJ, Mensah GA, Criqui MH. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet* 2013; **382**: 1329-1340 [PMID: 23915883 DOI: 10.1016/S0140-6736(13)61249-0]

17 **Arora E**, Maiya AG, Devasia T, Bhat R, Kamath G. Prevalence of peripheral arterial disease among type 2 diabetes mellitus in coastal Karnataka. *Diabetes Metab Syndr* 2019; **13**: 1251-1253 [PMID: 31336473 DOI: 10.1016/j.dsx.2019.02.003]

18 **Akalu Y**, Birhan A. Peripheral Arterial Disease and Its Associated Factors among Type 2 Diabetes Mellitus Patients at Debre Tabor General Hospital, Northwest Ethiopia. *J Diabetes Res* 2020; **2020**: 9419413 [PMID: 32090126 DOI: 10.1155/2020/9419413]

19 **Soyoye DO**, Ikem RT, Kolawole BA, Oluwadiya KS, Bolarinwa RA, Adebayo OJ. Prevalence and Correlates of Peripheral Arterial Disease in Nigerians with Type 2 Diabetes. *Adv Med* 2016; **2016**: 3529419 [PMID: 27800544 DOI: 10.1155/2016/3529419]

20 **Gregg EW**, Sorlie P, Paulose-Ram R, Gu Q, Eberhardt MS, Wolz M, Burt V, Curtin L, Engelgau M, Geiss L; 1999-2000 national health and nutrition examination survey. Prevalence of lower-extremity disease in the US adult population >=40 years of age with and without diabetes: 1999-2000 national health and nutrition examination survey. *Diabetes Care* 2004; **27**: 1591-1597 [PMID: 15220233 DOI: 10.2337/diacare.27.7.1591]

21 **Stoberock K**, Kaschwich M, Nicolay SS, Mahmoud N, Heidemann F, Rieß HC, Debus ES, Behrendt CA. The interrelationship between diabetes mellitus and peripheral arterial disease - a systematic review. *Vasa* 2020; 1-8 [PMID: 33175668 DOI: 10.1024/0301-1526/a000925]

22 **Wang N**, Yang BH, Wang G, Gao Y, Cao X, Zhang XF, Yan CC, Lian XT, Liu BH, Ju S. A meta-analysis of the relationship between foot local characteristics and major lower extremity amputation in diabetic foot patients. *J Cell Biochem* 2019; **120**: 9091-9096 [PMID: 30784095 DOI: 10.1002/jcb.28183]

23 **Beach KW**, Bedford GR, Bergelin RO, Martin DC, Vandenberghe N, Zaccardi M, Strandness DE Jr. Progression of lower-extremity arterial occlusive disease in type II diabetes mellitus. *Diabetes Care* 1988; **11**: 464-472 [PMID: 3402301 DOI: 10.2337/diacare.11.6.464]

24 **Costa RHR**, Cardoso NA, Procópio RJ, Navarro TP, Dardik A, de Loiola Cisneros L. Diabetic foot ulcer carries high amputation and mortality rates, particularly in the presence of advanced age, peripheral artery disease and anemia. *Diabetes Metab Syndr* 2017; **11** Suppl 2: S583-S587 [PMID: 28465149 DOI: 10.1016/j.dsx.2017.04.008]

25 **Wang A**, Sun X, Wang W, Jiang K. A study of prognostic factors in Chinese patients with diabetic foot ulcers. *Diabet Foot Ankle* 2014; **5** [PMID: 24765244 DOI: 10.3402/dfa.v5.22936]

26 **Adler AI**, Stevens RJ, Neil A, Stratton IM, Boulton AJ, Holman RR. UKPDS 59: hyperglycemia and other potentially modifiable risk factors for peripheral vascular disease in type 2 diabetes. *Diabetes Care* 2002; **25**: 894-899 [PMID: 11978687 DOI: 10.2337/diacare.25.5.894]

27 **Forrest KY**, Becker DJ, Kuller LH, Wolfson SK, Orchard TJ. Are predictors of coronary heart disease and lower-extremity arterial disease in type 1 diabetes the same? *Atherosclerosis* 2000; **148**: 159-169 [PMID: 10580182 DOI: 10.1016/s0021-9150(99)00217-8]

28 **Wilson PW**. Established risk factors and coronary artery disease: the Framingham Study. *Am J Hypertens* 1994; **7**: 7S-12S [PMID: 7946184 DOI: 10.1093/ajh/7.7.7s]

29 **Song P**, Rudan D, Zhu Y, Fowkes FJI, Rahimi K, Fowkes FGR, Rudan I. Global, regional, and national prevalence and risk factors for peripheral artery disease in 2015: an updated systematic review and analysis. *Lancet Glob Health* 2019; **7**: e1020-e1030 [PMID: 31303293 DOI: 10.1016/S2214-109X(19)30255-4]

30 **Aboyans V**, Criqui MH, Denenberg JO, Knoke JD, Ridker PM, Fronek A. Risk factors for progression of peripheral arterial disease in large and small vessels. *Circulation* 2006; **113**: 2623-2629 [PMID: 16735675 DOI: 10.1161/CIRCULATIONAHA.105.608679]

31 **Weragoda J**, Seneviratne R, Weerasinghe MC, Wijeyaratne SM. Risk factors of peripheral arterial disease: a case control study in Sri Lanka. *BMC Res Notes* 2016; **9**: 508 [PMID: 27938397 DOI: 10.1186/s13104-016-2314-x]

32 **Shukla V**, Fatima J, Ali M, Garg A. A Study of Prevalence of Peripheral Arterial Disease in Type 2 Diabetes Mellitus Patients in a Teaching Hospital. *J Assoc Physicians India* 2018; **66**: 57-60 [PMID: 30477058]

33 **Packard RR**, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. *Clin Chem* 2008; **54**: 24-38 [PMID: 18160725 DOI: 10.1373/clinchem.2007.097360]

34 **Fonseca V**, Desouza C, Asnani S, Jialal I. Nontraditional risk factors for cardiovascular disease in diabetes. *Endocr Rev* 2004; **25**: 153-175 [PMID: 14769830 DOI: 10.1210/er.2002-0034]

35 **Tzoulaki I**, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. Inflammatory, haemostatic, and rheological markers for incident peripheral arterial disease: Edinburgh Artery Study. *Eur Heart J* 2007; **28**: 354-362 [PMID: 17213229 DOI: 10.1093/eurheartj/ehl441]

36 **Shou Z**, Zhao Y, Zhang Y, Li S. Risk factors for peripheral arterial disease in elderly patients with Type-2 diabetes mellitus: A clinical study. *Pak J Med Sci* 2020; **36**: 1344-1348 [PMID: 32968406 DOI: 10.12669/pjms.36.6.2906]

37 **Aronow WS**, Ahn C. Association between plasma homocysteine and peripheral arterial disease in older persons. *Coron Artery Dis* 1998; **9**: 49-50 [PMID: 9589191 DOI: 10.1097/00019501-199809010-00008]

38 **Bembi V**, Singh S, Singh P, Aneja GK, Arya TV, Arora R. Prevalence of peripheral arterial disease in a cohort of diabetic patients. *South Med J* 2006; **99**: 564-569 [PMID: 16800410 DOI: 10.1097/01.smj.0000221624.68378.5d]

39 **Drexel H**, Aczel S, Marte T, Benzer W, Langer P, Moll W, Saely CH. Is atherosclerosis in diabetes and impaired fasting glucose driven by elevated LDL cholesterol or by decreased HDL cholesterol? *Diabetes Care* 2005; **28**: 101-107 [PMID: 15616241 DOI: 10.2337/diacare.28.1.101]

40 **American Diabetes Association**. . Peripheral arterial disease in people with diabetes. *Diabetes Care* 2003; **26**: 3333-3341 [PMID: 14633825 DOI: 10.2337/diacare.26.12.3333]

41 **Lee AY**, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J* 1999; **13**: 23-30 [PMID: 9872926 DOI: 10.1096/fasebj.13.1.23]

42 **Brownlee M**. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615-1625 [PMID: 15919781 DOI: 10.2337/diabetes.54.6.1615]

43 **Koya D**, Jirousek MR, Lin YW, Ishii H, Kuboki K, King GL. Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 1997; **100**: 115-126 [PMID: 9202063 DOI: 10.1172/JCI119503]

44 **Geng YJ**, Libby P. Progression of atheroma: a struggle between death and procreation. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1370-1380 [PMID: 12231554 DOI: 10.1161/01.atv.0000031341.84618.a4]

45 **Aboyans V**, Björck M, Brodmann M, Collet JP, Czerny M, De Carlo M, Naylor AR, Roffi M, Tendera M, Vlachopoulos C, Ricco JB; ESC Scientific Document Group. Questions and answers on diagnosis and management of patients with Peripheral Arterial Diseases: a companion document of the 2017 ESC Guidelines for the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Endorsed by: the European Stroke Organisation (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). *Eur Heart J* 2018; **39**: e35-e41 [PMID: 29088383 DOI: 10.1093/eurheartj/ehx499]

46 **Hennion DR**, Siano KA. Diagnosis and treatment of peripheral arterial disease. *Am Fam Physician* 2013; **88**: 306-310 [PMID: 24010393]

47 **Doobay AV**, Anand SS. Sensitivity and specificity of the ankle-brachial index to predict future cardiovascular outcomes: a systematic review. *Arterioscler Thromb Vasc Biol* 2005; **25**: 1463-1469 [PMID: 15879302 DOI: 10.1161/01.ATV.0000168911.78624.b7]

48 **Gerhard-Herman MD**, Gornik HL, Barrett C, Barshes NR, Corriere MA, Drachman DE, Fleisher LA, Fowkes FG, Hamburg NM, Kinlay S, Lookstein R, Misra S, Mureebe L, Olin JW, Patel RA, Regensteiner JG, Schanzer A, Shishehbor MH, Stewart KJ, Treat-Jacobson D, Walsh ME. 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* 2017; **135**: e686-e725 [PMID: 27840332 DOI: 10.1161/CIR.0000000000000470]

49 **Rooke TW**, Hirsch AT, Misra S, Sidawy AN, Beckman JA, Findeiss L, Golzarian J, Gornik HL, Jaff MR, Moneta GL, Olin JW, Stanley JC, White CJ, White JV, Zierler RE; American College of Cardiology Foundation Task Force; American Heart Association Task Force. Management of patients with peripheral artery disease (compilation of 2005 and 2011 ACCF/AHA Guideline Recommendations): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2013; **61**: 1555-1570 [PMID: 23473760 DOI: 10.1016/j.jacc.2013.01.004]

50 **Lane R**, Harwood A, Watson L, Leng GC. Exercise for intermittent claudication. *Cochrane Database Syst Rev* 2017; **12**: CD000990 [PMID: 29278423 DOI: 10.1002/14651858.CD000990.pub4]

51 **McDermott MM**. Exercise Rehabilitation for Peripheral Artery Disease: A REVIEW. *J Cardiopulm Rehabil Prev* 2018; **38**: 63-69 [PMID: 29465495 DOI: 10.1097/HCR.0000000000000343]

52 **Piepoli MF**, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corrà U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs FDR, Løchen ML, Löllgen H, Marques-Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, van der Worp HB, van Dis I, Verschuren WMM, Binno S; ESC Scientific Document Group. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European

Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016; **37**: 2315-2381 [PMID: 27222591 DOI: 10.1093/eurheartj/ehw106]

53 **Reiter-Brennan C**, Osei AD, Iftekhar Uddin SM, Orimoloye OA, Obisesan OH, Mirbolouk M, Blaha MJ, Dzaye O. ACC/AHA lipid guidelines: Personalized care to prevent cardiovascular disease. *Cleve Clin J Med* 2020; **87**: 231-239 [PMID: 32238379 DOI: 10.3949/ccjm.87a.19078]

54 **Murphy SA**, Cannon CP, Blazing MA, Giugliano RP, White JA, Lokhnygina Y, Reist C, Im K, Bohula EA, Isaza D, Lopez-Sendon J, Dellborg M, Kher U, Tershakovec AM, Braunwald E. Reduction in Total Cardiovascular Events With Ezetimibe/Simvastatin Post-Acute Coronary Syndrome: The IMPROVE-IT Trial. *J Am Coll Cardiol* 2016; **67**: 353-361 [PMID: 26821621 DOI: 10.1016/j.jacc.2015.10.077]

55 **Sabatine MS**, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR; FOURIER Steering Committee and Investigators. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. *N Engl J Med* 2017; **376**: 1713-1722 [PMID: 28304224 DOI: 10.1056/NEJMoa1615664]

56 **Berger JS**, Krantz MJ, Kittelson JM, Hiatt WR. Aspirin for the prevention of cardiovascular events in patients with peripheral artery disease: a meta-analysis of randomized trials. *JAMA* 2009; **301**: 1909-1919 [PMID: 19436018 DOI: 10.1001/jama.2009.623]

57 **CAPRIE Steering Committee**. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet* 1996; **348**: 1329-1339 [PMID: 8918275 DOI: 10.1016/S0140-6736(96)09457-3]

58 **Hiatt WR**, Fowkes FG, Heizer G, Berger JS, Baumgartner I, Held P, Katona BG, Mahaffey KW, Norgren L, Jones WS, Blomster J, Millegård M, Reist C, Patel MR; EUCLID Trial Steering Committee and Investigators. Ticagrelor versus Clopidogrel in Symptomatic Peripheral Artery Disease. *N Engl J Med* 2017; **376**: 32-40 [PMID: 27959717 DOI: 10.1056/NEJMoa1611688]

59 **Robless P**, Mikhailidis DP, Stansby GP. Cilostazol for peripheral arterial disease. *Cochrane Database Syst Rev* 2008; CD003748 [PMID: 18254032 DOI: 10.1002/14651858.CD003748.pub3]

60 **Diabetes Control and Complications Trial Research Group**, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]

61 **Nathan DM**, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; **353**: 2643-2653 [PMID: 16371630 DOI: 10.1056/NEJMoa052187]

62 Professional Practice Committee: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020; **43**: S3 [PMID: 31862742 DOI: 10.2337/dc20-Sppc]

63 **Holman RR**, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008; **359**: 1577-1589 [PMID: 18784090 DOI: 10.1056/NEJMoa0806470]

64 **Skyler JS**, Bergenstal R, Bonow RO, Buse J, Deedwania P, Gale EA, Howard BV, Kirkman MS, Kosiborod M, Reaven P, Sherwin RS; American Diabetes Association; American College of Cardiology Foundation; American Heart Association. Intensive glycemic control and the prevention of cardiovascular events: implications of the ACCORD, ADVANCE, and VA diabetes trials: a position statement of the American Diabetes Association and a scientific statement of the American College of Cardiology Foundation and the American Heart Association. *Diabetes Care* 2009; **32**: 187-192 [PMID: 19092168 DOI: 10.2337/dc08-9026]

65 **Zoungas S**, Chalmers J, Neal B, Billot L, Li Q, Hirakawa Y, Arima H, Monaghan H, Joshi R, Colagiuri S, Cooper ME, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Lisheng L, Mancia G, Marre M, Matthews DR, Mogensen CE, Perkovic V, Poulter N, Rodgers A, Williams B, MacMahon S, Patel A, Woodward M; ADVANCE-ON Collaborative Group. Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. *N Engl J Med* 2014; **371**: 1392-1406 [PMID: 25234206 DOI: 10.1056/NEJMoa1407963]

66 **Action to Control Cardiovascular Risk in Diabetes Study Group**, Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH Jr, Probstfield JL, Simons-Morton DG, Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008; **358**: 2545-2559 [PMID: 18539917 DOI: 10.1056/NEJMoa0802743]

67 **Hayward RA**, Reaven PD, Wiitala WL, Bahn GD, Reda DJ, Ge L, McCarren M, Duckworth WC, Emanuele NV; VADT Investigators. Follow-up of glycemic control and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2015; **372**: 2197-2206 [PMID: 26039600 DOI: 10.1056/NEJMoa1414266]

68 **Williams B**, Mancia G. Ten Commandments of the 2018 ESC/ESH HTN Guidelines on Hypertension in Adults. *Eur Heart J* 2018; **39**: 3007-3008 [PMID: 30990869 DOI: 10.1093/eurheartj/ehy439]

69 **Margolis KL**, O'Connor PJ, Morgan TM, Buse JB, Cohen RM, Cushman WC, Cutler JA, Evans GW, Gerstein HC, Grimm RH Jr, Lipkin EW, Narayan KM, Riddle MC Jr, Sood A, Goff DC Jr. Outcomes of combined cardiovascular risk factor management strategies in type 2 diabetes: the ACCORD randomized trial. *Diabetes Care* 2014; **37**: 1721-1728 [PMID: 24595629 DOI: 10.2337/dc13-2334]

70 **Ontarget Investigators**, Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P, Anderson C. Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008; **358**: 1547-1559 [PMID: 18378520 DOI: 10.1056/NEJMoa0801317]

71 **Heart Outcomes Prevention Evaluation Study Investigators**, Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000; **342**: 145-153 [PMID: 10639539 DOI: 10.1056/NEJM200001203420301]

72 **Pereira K**. Treatment Strategies for the Claudicant. *Semin Intervent Radiol* 2018; **35**: 435-442 [PMID: 30728659 DOI: 10.1055/s-0038-1676322]

73 **Lazar A**, Morrissey N. Recent advances in endovascular treatment of peripheral arterial disease. *F1000Res* 2020; **9** [PMID: 32148765 DOI: 10.12688/f1000research.20398.1]

74 **Topfer LA**, Spry C. New Technologies for the Treatment of Peripheral Artery Disease. 2018 Apr 1. In: CADTH Issues in Emerging Health Technologies. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2016–. 172 [PMID: 30148583]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 839-854

DOI: 10.4239/wjd.v12.i6.839

ISSN 1948-9358 (online)

MINIREVIEWS

New perspectives on angiotensin-converting enzyme 2 and its related diseases

Li-Ping Liu, Xiao-Li Zhang, Jian Li

ORCID number: Li-Ping Liu 0000-0003-1738-6125; Xiao-Li Zhang 0000-0003-0076-0854; Jian Li 0000-0002-6210-7415.

Author contributions: Liu LP and Zhang XL wrote the manuscript; Li J designed and revised the review.

Supported by National Natural Science Foundation of China, No. 81873861; and Key Grant of Research and Development in Hunan Province, No. 2020DK2002.

Conflict-of-interest statement: The authors declare no conflicts of interest related to this manuscript.

Manuscript source: Invited manuscript

Li-Ping Liu, Jian Li, Key Laboratory of Model Animals and Stem Cell Biology in Hunan Province, School of Medicine, Hunan Normal University, Changsha 410013, Hunan Province, China

Xiao-Li Zhang, TheFifth Department of Medicine (Nephrology/Endocrinology/Rheumatology), University Medical Centre Mannheim, University of Heidelberg, Heidelberg 68135, Baden-Württemberg, Germany

Corresponding author: Jian Li, PhD, Associate Professor, Key Laboratory of Model Animals and Stem Cell Biology in Hunan Province, School of Medicine, Hunan Normal University, No. 371 Tongzhipo Road, Changsha 410013, Hunan Province, China. lijianyxy@hunnu.edu.cn

Abstract

Since the worldwide outbreak of coronavirus disease 2019, angiotensin-converting enzyme 2 (ACE2) has received widespread attention as the cell receptor of the severe acute respiratory syndrome coronavirus 2 virus. At the same time, as a key enzyme in the renin-angiotensin-system, ACE2 is considered to be an endogenous negative regulator of vasoconstriction, proliferation, fibrosis, and proinflammation caused by the ACE-angiotensin II-angiotensin type 1 receptor axis. ACE2 is now implicated as being closely connected to diabetes, cardiovascular, kidney, and lung diseases, and so on. This review covers the available information on the host factors regulating ACE2 and discusses its role in a variety of pathophysiological conditions in animal models and humans.

Key Words: Angiotensin-converting enzyme 2; COVID-19; Salt; Renin-angiotensin-system inhibitors; Diabetes and cardiovascular disease; Renal and lung disease

Core Tip: Angiotensin-converting enzyme 2 (ACE2) as the key cell receptor for the severe acute respiratory syndrome coronavirus 2 virus has received widespread attention. This paper will review the new perspectives on ACE2, covers available information on the host regulative factors of ACE2, and discusses its role in a variety of pathophysiological conditions. This review will help us with a better understanding of the biological function and role of ACE2 in coronavirus disease 2019 and its

Specialty type: Medicine, research and experimental

Country/Territory of origin: China

Peer-review report's scientific quality classification

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

Received: February 18, 2021
Peer-review started: February 18, 2021
First decision: March 16, 2021
Revised: March 30, 2021
Accepted: April 20, 2021
Article in press: April 20, 2021
Published online: June 15, 2021

P-Reviewer: Manenti A
S-Editor: Yan JP
L-Editor: Wang TQ
P-Editor: Ma YJ

treatment.

Citation: Liu LP, Zhang XL, Li J. New perspectives on angiotensin-converting enzyme 2 and its related diseases. *World J Diabetes* 2021; 12(6): 839-854
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/839.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.839

INTRODUCTION

Angiotensin-converting enzyme 2 (ACE2), as the key enzyme in the renin angiotensin system (RAS) and key cell receptor for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, has received considerable attention[1] since coronavirus disease 2019 (COVID-19) has spread worldwide. Patients with diabetes and renal and cardiovascular diseases are widely treated with ACE inhibitors (ACEIs)/angiotensin II receptor blockers (ARBs), which are deemed to potentially increase ACE2 expression in the body; the consequent increased expression of ACE2 could facilitate infection with SARS-CoV-2. The paradox of the use of ACEI/ARB treatment to interfere with the RAS because ACE2 reduces inflammation (which has been suggested as a therapy for inflammatory lung diseases, diabetes, and hypertension) and the probable contribution of ACE2 to an increased risk of COVID-19 have puzzled clinicians and attracted much attention[2,3]. Therefore, it is necessary to further understand the characteristics of ACE2 and accurate details of the molecular mechanisms of ACE2 that underlie these phenomena. This review will focus on the biological function of ACE2 and its host regulatory factors. Moreover, we will discuss the role of ACE2 in a variety of pathophysiological conditions, including cardiovascular, kidney, and lung diseases and diabetes in animal models and humans. We aim to provide new perspectives on ACE2 and help us better understand the role of ACE2 in SARS-CoV-2 infection and its treatment nowadays (Figure 1).

BIOCHEMICAL CHARACTERISTICS AND DISTRIBUTION OF ACE2

ACE2 is a type I membrane protein that includes an N-terminal peptidase domain (PD) and a C-terminal collectrin-like domain. The PD of ACE2 provides a direct binding site for the S protein of coronavirus[4]. ACE2 was discovered in 2000 when Tipnis *et al*[5] and Donoghue *et al*[6] cloned ACE2 from a human heart failure (HF) cDNA library and a human lymphoma cDNA library and found to be a homolog of the ACE gene. The gene is located in the Xp22 region and has a total length of approximately 39.98 kb, including 18 exons and 17 introns. ACE2 can be hydrolyzed by depolymerase into soluble ACE2, and therefore, ACE2 has two forms, the membrane-linked type and the soluble type. Membrane-linked ACE2 is an extracellular enzyme that is distributed on the cell membrane surface and consists of four parts: The N-terminal signal peptide region, the zinc binding motif (amino acid residues 374 to 378), the transmembrane region (amino acids 740 to 768), and the C-terminal intracellular domain. Dissolved ACE2 is distributed mainly in the plasma and urine due to the lack of a transmembrane region and a C-terminal intracellular domain[5]. ACE2 is widely expressed in many organs, including the heart, kidney, testis, adipose tissue, brain tissue, vascular smooth muscle cells, gastrointestinal tract, and lung (type 2 alveolar epithelial cells) and is highly specific in tissues[7].

FUNCTIONS OF ACE2

ACE2 is a key enzyme in the RAS and is considered to be an endogenous negative regulator of vasoconstriction, proliferation, fibrosis, and proinflammation caused by the ACE-angiotensin II (Ang II)-angiotensin type 1 receptor (AT1R) axis[8]. In the RAS, ACE cuts Ang I to convert it into Ang II, and then Ang II binds to the G protein-coupled receptor AT1R, which causes vasoconstriction and increased blood pressure [9]. The function of ACE2 is opposite to that of ACE. ACE2 cuts Ang II and converts it

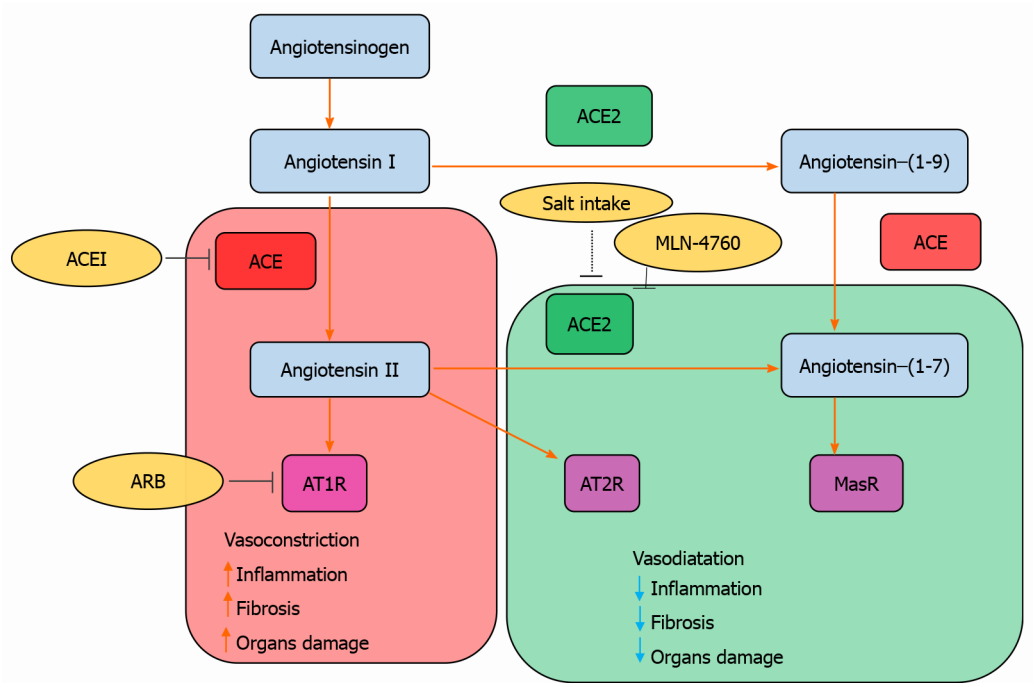

Figure 1 Composition and function of the renin-angiotensin system and its main regulators and inhibitors. RAS: Rennin angiotensin system; ACEI: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin II receptor blocker; ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme 2; AT1R: Angiotensin type 1 receptor; AT2R: Angiotensin type 2 receptor; MasR: Mas receptor; MLN-4760: A specific ACE2 inhibitor; Organ damage: Renal damage, lung damage, cardiovascular damage, *etc.*

into angiotensin 1-7 (Ang 1-7), which can lower blood pressure[10]. Increasing evidence demonstrates that ACE2 plays an important role in a variety of pathophysiological conditions. In the tumor microenvironment, ACE2 acts on Ang II to produce endogenous Ang 1-7. Ang 1-7 binds to the Mas receptor (MasR) and exerts antitumor effects such as antiproliferation, antiangiogenesis, anti-invasion, and antimetastasis effects through a series of signal transduction pathways. In diabetes, upregulation of ACE2 can improve hyperglycemia, and Ang 1-7 can improve metabolic syndrome through glucose intake and oxidative stress related to insulin resistance[11]. The expression of ACE2 is related to atherosclerosis[12]. The overexpression of ACE2 can improve endothelial-dependent vascular relaxation, increase the proliferative activity of endothelial cells, and facilitate the migration of endothelial cells. ACE2 exerts anti-infective properties through Ang 1-7[13]. The decrease in aortic ACE2 expression increases the expression of proinflammatory factors, such as tumor necrosis factor α, interleukin-6, monocyte chemotactic protein 1, vascular cell adhesion molecule 1, matrix metalloproteinase (MMP)-2 and MMP-9, which are conducive to the adhesion of leukocytes to endothelial cells and blood vessel walls. In addition, ACE2 can also regulate the adhesion of macrophages to endothelial cells[14]. The combination of Ang 1-7 and MasR can also inhibit the formation of thrombi[15] and liver fibrosis[16]. In ischemic stroke, Ang 1-7 can reduce the area of cerebral infarction and brain dysfunction by regulating the release of nitric oxide from different sources to protect the brain. In addition, Ang 1-7 in brain tissue can regulate learning and memory functions. In the reproductive system, Ang 1-7 can regulate endometrial function, spermatogenesis, follicular maturation, ovulation, and pregnancy-related processes[11]. ACE2 can also regulate the immunity of intestinal epithelial cells through amino acid homeostasis, expression of antimicrobial peptides, and regulation of the balance of intestinal microbes[17].

ACE2 AND SALT

It has been widely demonstrated that a high-sodium (HS) diet activates the RAS. Animal experiments have proven that salt intake is a powerful regulator of ACE2 expression in animal models[18]. Studies by Samuel *et al*[19] found that the relative expression patterns of ACE and AT1R increased, renin levels decreased, and ACE2, AT2R, and MasR remained unaltered in HS-fed lean Zucker rats. On the other hand,

HS intake caused an increase in the cortical expression of ACE and a decrease in ACE2, accompanied by increased blood pressure. Elevated blood pressure is associated with a significant increase in Ang II levels in the renal cortex in obese rats and a decrease in the expression of the ACE2-AT2R-MasR axis in obese Zucker rats. In a study of a left nephrectomy rat model[20], HS diet intake increased the glomerular ACE/ACE2 ratio, which was associated with decreased ACE2. In ACE2 KO mice, ACE2 deficiency significantly increased renal oxidative stress by reducing the production of Ang 1-7. In another study, the consumption of a HS diet in normotensive animals reduced ACE2 protein expression and raised renal oxidative stress[21]. Another study showed that the ACE/ACE2 protein ratio was increased in the kidneys of spontaneously hypertensive rats (SHRs) when fed diets with high levels of NaCl. At the same time, compared to the other SHR groups, there was an increase in kidney ACE2 protein and activity in the group fed a long-term low salt diet[18]. Similarly, Varagic *et al*[22] used an SHR model to prove that HS intake decreased cardiac ACE2 mRNA and protein expression. In Dahl salt-sensitive hypertensive rats, a HS diet reduced ACE2 mRNA expression and augmented the local RAS, which induced hypertension[23]. The HS diet also decreased ACE2 protein expression as assessed using immunohistochemistry compared to a normal-salt diet[24].

In summary, these findings show that a HS diet can reduce the expression of ACE2, thereby affecting the RAS. Further studies have reported that interventions that augment ACE2 expression or activity can be helpful to prevent cardiovascular damage [25]. Therefore, elucidating the precise mechanisms involved in the interaction of sodium intake and ACE2 will be conducive to predicting the physiological and pathological changes caused by a HS diet (Table 1).

ACE2 AND RAS INHIBITORS

Some studies in animal models have demonstrated that both ACEIs (lisinopril, enalapril, and ramipril) and ARBs (losartan, olmesartan, and telmisartan) can upregulate ACE2 expression. Ferrario *et al*[26] showed that *ACE2* mRNA expression increased in the left ventricle of normotensive Lewis rats after 12 d of lisinopril or losartan treatment. Lisinopril increased ACE2 levels by 5-fold, while losartan increased ACE2 levels by 3-fold. A rat study from Ocaranza *et al*[27] showed that enalapril prevented the decrease in mRNA levels and activities of ACE2 in late ventricular dysfunction after myocardial infarction. The results of Ishiyama *et al*[28] indicated that the level of *ACE2* mRNA increased after ARB treatment in rats with coronary artery ligation. A mouse study by Soler *et al*[29] showed that ACE2 is preferentially localized in the tunica media of kidney arterioles, and its expression is amplified after administration of telmisartan. On the other hand, some association studies have shown no increase in *ACE2* mRNA after ACEI or ARB treatment. An animal study by Burrell *et al*[30] found that ramipril attenuated cardiac hypertrophy and inhibited cardiac ACE but had no effect on cardiac *ACE2* mRNA in rats after coronary artery ligation. Subsequent studies by Burchill *et al*[31] provided evidence that there was no increase in ACE2 mRNA or protein expression in rats after coronary artery ligation and treatment with valsartan, ramipril, or both when compared to the control group. In general, these studies on experimental animals did not provide consistent evidence to prove the effect of ARB/ACEI administration on ACE2 protein expression. In the case of simulating human drug delivery, further experimental studies are needed.

With the pandemic of COVID-19 spreading since January 2020, it has been inferred that increased ACE2 expression in the lungs correlated with a higher risk of SARS-CoV-2 infection in patients with cardiac and renal diseases, hypertension, and diabetes treated with ACEIs or ARBs. However, a review from Sriram and Insel[32] showed that there was no clear evidence that elevated ACE2 expression when using ACEIs/ARBs could increase the risk of SARS-CoV-2 infection. In a study of 362 hospitalized hypertensive COVID-19 patients, there was no significant difference between severe and noncritical patients or between non-survivors and survivors according to the use of ACEIs and/or ARBs[33]. Conversely, in a recent meta-analysis from Chu *et al*[34], it was suggested that ACEI treatment reduced the risk of infection with SARS-CoV-2 and that blocking the RAS might decrease all-cause mortality in COVID-19 patients. This study also reported that ACEIs reduced the risk of non-COVID pneumonia and all-cause mortality caused by non-COVID pneumonia. The effect of ACEIs/ARBs on the expression of ACE2 and the effect on human infection with SARS-CoV-2 remain unknown and complex. Some research has supported the hypothesis

Table 1 List of animal studies on the high-sodium diet and angiotensin-converting enzyme 2

Animal	Study details	Main findings	Ref.
Zucker rats	Lean and obese Zucker rats were fed a normal-sodium diet (0.4%) or a high-sodium diet (8%) for 2 wk	ACE2 mRNA and protein expression was significantly reduced in HS-fed obese Zucker rats	Samuel *et al* [19]
Wistar rats	Rats were fed three salt concentrations (0.2%, 1.2%, 8.2%) for 4 wk after uninephrectomy of the left kidney	HS diet increased the glomerular ACE/ACE2 ratio	Bernardi *et al*[20]
Wistar rats	After 4 wk of induction of hypertension (2-kidney 1-clip model), rats were fed a normal-sodium diet (0.4%) or a high-sodium diet (8%) for 2 wk	HS diet decreased urinary angiotensinogen, ACE, and ACE2 expression in the clipped and unclipped kidneys	Shimoura *et al*[21]
SHR	Rats were fed three NaCl content diets (0.03%, 0.3%, 3%) for 6 mo	HS diet decreased ACE2 protein expression in kidney tissues	Berger *et al* [18]
SHR	Rats were fed an 8% salt diet for 5 wk	HS diet decreased cardiac ACE2 mRNA and protein levels	Varagic *et al*[22]
DS and DR rats	DS and DR rats were fed low-sodium chow (0.45%) or high-sodium chow (7%) for 8 wk and treated with or without eplerenone (100 mg/kg/d), candesartan (10 mg/kg/d), or both drugs for 8 wk	HS diet increased angiotensinogen mRNA and decreased *ACE2* mRNA in the hearts of DS rats; candesartan increased *ACE2* mRNA levels in the heart	Takeda *et al* [23]
SD rats	Rats were fed an 8% NaCl high-salt or 0.4% NaCl (normal-salt) diet for 3 wk, with or without antioxidant supplementation with tempol	HS diet decreased ACE2 expression; tempol reversed the imbalance of renal RAS components (decrease in Ang II and AT1R and increase in AT2, ACE2, Ang 1-7, and MasR staining intensity)	Cao *et al* [24]

SHR: Spontaneously hypertensive rats; DS rats: Dahl salt-sensitive hypertensive rats; DR rats: Dahl salt-resistant rats; SD rats: Sprague-Dawley rats; HS: High-sodium; ACE2: Angiotensin-converting enzyme 2; MasR: Mas receptor; AT1R: Angiotensin type 1 receptor; AT2: Angiotensin type 2; Ang 1-7: Angiotensin 1-7; RAS: Renin angiotensin system.

that ACE inhibition by ACEIs might stimulate negative feedback, upregulating ACE2 expression but decreasing overall inflammation in the absence of angiotensin II[35]. Thus, the effects of ACEIs/ARBs vary depending on the clinical stage: Negative in the initial infection phase but positive in the tissue inflammation stage[36,37]. Most researchers believe that the use of ARB or ACEI drugs should not be stopped for the purpose of reducing SARS-CoV-2 infection. Stopping maintenance treatment may cause blood pressure imbalance or HF. The guidelines should be revised quickly based on various clinical data, and personalized treatment should be carried out in accordance with clinical manifestations[38] (Table 2).

ACE2 AND DISEASES

The functional components of the RAS are in balance with each other to maintain the health of the body. Under certain pathological conditions, Ang II, AT1R, or ACE levels can increase or become unbalanced, which is detrimental. It has been two decades since the discovery of ACE2. During this period, efforts towards the characterization of this enzyme have provided greater insights into the RAS. The ongoing studies provoked more questions, particularly regarding the role of ACE2 in the development and progression of hypertension and renal injury, as well as other pathologies, including diabetes, HF, liver fibrosis, and lung injury (Tables 3 and 4).

ACE2 and its role in the kidney and diabetes

The kidney possesses a fully functional local RAS capable of producing Ang II, a major contributor to the progression of chronic kidney disease. ACE2 is highly expressed in the kidney and predominantly localized to proximal tubules and glomerular podocytes[39,40]. Several lines of evidence indicate that ACE2 serves as a key protective enzyme to prevent progressive renal damage by reducing oxidative stress, inflammation, and fibrosis[41-43].

The study of ACE2 in the context of diabetes has focused primarily on the kidney. ACE2 may be an important target for the treatment and prevention of diabetic nephropathy (DN). ACE2 expression in the kidney has been studied in both type 1 diabetes (T1D) and type 2 diabetes (T2D) models. The majority of animal studies indicate that ACE2 expression is downregulated in the glomeruli in diabetes, whereas tubular ACE2 expression is upregulated[41,44-46]. Tikellis *et al*[47] first reported that ACE2 expression was reduced in the kidneys of rats with longstanding diabetes

Table 2 List of animal studies on renin angiotensin system inhibitors and angiotensin-converting enzyme 2

Animal	Model	Study details	Main findings	Ref.
Lewis rats	Normotensive	Rats were assigned to drink water containing losartan or lisinopril at 10 mg/kg/d for 12 d	Lisinopril or losartan increased cardiac *ACE2* mRNA, but the combination did not produce this effect	Ferrario *et al* [26]
SD rats	MI	Enalapril was given to rats after sham operation or LCA ligation	Enalapril prevented the decrease of ventricular *ACE2* mRNA levels and activities post-MI	Ocaranza *et al*[27]
Lewis rats	MI	Losartan and olmesartan was administered for 28 d after coronary artery ligation	The level of *ACE2* mRNA increased after treatment with losartan and olmesartan	Ishiyama *et al*[28]
C57BLKS/J mice	Normotensive	Mice were treated with telmisartan at 2 mg/kg/d for 2 wk	Telmisartan increased ACE2 expression in the tunica media of renal arterioles	Soler *et al*[29]
SD rats	MI	SD rats received ramipril at 1 mg/kg/d for 28 d after MI operation	Ramipril inhibited cardiac ACE but had no effect on cardiac *ACE2* mRNA after MI	Burrell *et al* [30]
SD rats	MI	Ramipril (1 mg/kg/d) and valsartan (10 mg/kg/d) were given for 28 d after MI operation	Cardiac ACE2 expression was not augmented after either treatment alone or in combination	Burchill *et al* [31]

MI: Myocardial infarction; LCA: Left ventricular artery; SD rats: Sprague-Dawley rats; ACE2: Angiotensin-converting enzyme 2.

mellitus. In 8-wk-old db/db mice, a model of early T2D, ACE2 expression is elevated, while ACE expression is decreased in both glomeruli and the cortex[48] prior to the development of DN. In another study of db/db mice, Chodavarapu *et al*[45] demonstrated that the protein expression of ACE2 was reduced in glomeruli, while tubular ACE2 and a disintegrin and metalloprotease 17 were increased. In two models of T1D [streptozotocin (STZ)-induced and Akita mouse (Ins2WT/C96Y) models], *ACE2* gene deletion accelerated the development of DN[49,50], which could be ameliorated by perindopril or irbesartan. Moreover, treatment with recombinant human ACE2 (rhACE2) in male Akita mice led to reductions in albuminuria, hypertension, plasma Ang II levels, activation of NADPH oxidase, glomerular hypertrophy, and mesangial matrix expansion, thereby preventing the progression of DN[51]. In another rat study, the injection of adenoviral (Ad)-ACE2 in STZ-induced diabetic rats for 4 wk improved many signs of DN[52]. Furthermore, Ad-ACE2 and ACEI had similar effects, whereas the combined use of Ad-ACE2 and ACEI offered no additional benefits[52].

In humans, the expression of ACE2 was significantly reduced in both the glomeruli and proximal tubules in biopsy samples collected from patients with T2D-induced kidney disease[53]. Conversely, in a real-time polymerase chain reaction study, *ACE2* mRNA expression was not significantly changed in eight diabetic patients with overt proteinuria compared with 66 nondiabetic patients with renal disease[54]. The differences in the results obtained in human studies of T2D nephropathy might be due to the different stages of diabetes. To date, no human studies of early-stage diabetes have been conducted. Moreover, genetic variation in and around the gene encoding ACE2 is most often detected using single nucleotide polymorphisms (SNPs). In a recent study, 14 *ACE2* polymorphisms were genotyped by matrix-assisted laser desorption ionization time-of-flight mass spectrometry in the Uygur population of the Xinjiang region of China. Among them, the *ACE2* SNPs rs2074192, rs4240157, rs4646188, and 879922 were associated with increased microalbuminuria in T2D patients[55].

Taken together, the above studies suggest that ACE2 might play a protective role against the development of DN.

ACE2 and hypertension

The role of ACE2 has been intensively studied in models of hypertension. Crackower *et al*[56] first reported that ACE2 transgenic mice exhibited lower blood pressure than wild-type mice. Subsequent studies reported that ACE2 probably has a small effect on blood pressure in mice under normal conditions[57-59]. However, it plays a much more prominent role in the regulation of hypertension, especially when Ang II levels are elevated. Existing studies have shown that ACE2 was reduced in kidneys from rat models of hypertension, such as salt-sensitive Sabra hypertensive rats, SHRs, and stroke-prone SHRs (SHRSP)[56,60]. Moreover, in a sheep model of fetal programmed hypertension, the administration of betamethasone on the 80th day of gestation markedly reduced ACE2 activity in the proximal tubules and urine in adolescent sheep[61]. Rentzsch *et al*[62] assessed the role of ACE2 in the pathogenesis of

Table 3 List of animal studies on the role of angiotensin-converting enzyme 2 in diabetes, hypertension, cardiovascular disease, and acute lung injury

Disease	Animal	Study details	Main findings	Ref.
Diabetes	db/db mice	Mice were randomly assigned to four treatment groups: (1) Control group fed normal chow; (2) Control group fed rosiglitazone diet; (3) db/db group fed normal chow; and (4) db/db group fed rosiglitazone diet	Protein expression of glomerular ACE2 was decreased in the kidneys of db/db mice, while tubular ACE2 and ADAM17 were increased. Rosiglitazone treatment of db/db mice normalized hyperglycemia, attenuated renal injury, and decreased urinary ACE2 and renal ADAM17 protein expression	Chodavarapu *et al*[45]
	db/db mice	Mice were treated for 16 wk with a specific ACE2 inhibitor (MLN-4760) alone or combined with telmisartan.	ACE and ACE2 colocalized on the apical surface of the proximal tubules, whereas in glomeruli, ACE2 is present in podocytes and, to a lesser extent, in glomerular mesangial cells, whereas ACE is present only in endothelial cells. Telmisartan prevented the increase in UAE associated with the ACE2 inhibitor	Ye *et al*[46]
	db/db mice	ACE and ACE 2 expression was measured in the kidney and heart	ACE2 protein in renal cortical tubules was increased, whereas ACE protein was decreased. In heart tissue, there were no significant differences between db/db and db/m mice in either ACE or ACE2 expression	Ye *et a l*[48]
	STZ-induced diabetic SD rats	ACE2 and ACE gene and protein expression was measured in the kidney	*ACE2* and *ACE* mRNA levels were decreased in diabetic renal tubules by approximately 50% and were not influenced by ramipril	Tikellis *et al* [47]
	STZ-induced diabetic Wistar rats	Diabetic Wistar rats were treated with DIZE	Treatment with DIZE restored ACE2 expression in glomeruli and increased the expression of AT2 receptors in whole kidney and isolated glomeruli	Goru *et al*[44]
	Akita and $Ace2^{-/-}$ mice	$Ace2^{-/-}$ mice were crossed with Akita mice ($Ins^{2WT/C96Y}$), and four groups of mice were studied: $Ace2^{+/y}Ins2^{WT/WT}$, $Ace2^{-/y}Ins2^{WT/WT}$, $Ace2^{+/y}Ins2^{WT/C96Y}$, and $Ace2^{-/y}$ $Ins2^{WT/C96Y}$. The $Ace2^{+/y}$ $Ins2^{WT/C96Y}$ and $Ace2^{-/y}$ $Ins2^{WT/C96Y}$ mice were treated with the ARB (irbesartan)	Deletion of the *ACE2* gene was associated with accelerated kidney injury and reduced ACE2 expression in diabetic mice. Irbesartan reduced urinary albumin excretion rate in $Ace2^{-/y}$ $Ins2^{WT/C96Y}$ mice	Wong *et al*[49]
	STZ-induced diabeticC57BL/6J mice and ACE2 knockout (KO) mice	Control and diabetic C57BL/6J and ACE2 KO mice, after 5 wk without treatment, were randomized to receive the ACE inhibitor perindopril. Wild-type mice were further randomized to receive the selective ACE2 inhibitor MLN-4760	Induction of diabetes in wild-type mice was associated with a reduction in renal ACE2 expression and decreased Ang 1-7. In diabetic mice receiving MLN-4760 and in ACE2 KO mice, diabetes-associated albuminuria was enhanced	Tikellis *et al* [50]
	Akita mice	Male diabetic Akita mice ($Ins2^{(WT/C96Y)}$) and control C57BL/6J mice ($Ins2^{(WT/WT)}$) were injected daily with placebo or with rhACE2 (2 mg/kg) for 4 wk	Treatment with rhACE2 increased plasma ACE2 activity, normalized blood pressure, and reduced the urinary albumin excretion	Oudit *et al*[51]
	STZ-induced diabetic Wistar rats	Diabetic Wistar rats were divided into 5 groups: No-treatment group, adenoviral (Ad)-ACE2 group, Ad-green fluorescent protein (GFP) group, ACEI group receiving benazepril and Ad-ACE2 + ACEI group	Rats in Ad-ACE2 group exhibited reduced SBP, urinary albumin excretion, creatinine clearance, glomeruli sclerosis index, and renal malondialdehyde level; downregulated transforming growth factor (TGF)-β1, vascular endothelial growth factor (VEGF), and collagen IV protein expression; and increased renal superoxide dismutase activity. Ad-ACE2 and ACEI had similar effects, whereas combined use of Ad-ACE2 and ACEI offered no additional benefits	Liu *et al*[52]
Hypertension	Salt-sensitive Sabra hypertensive rats, SHR, and SHRSP	ACE2 expression levels were determined in the kidneys	ACE2 levels were reduced in all of these hypertensive rat strains	Crackower *et al*[56]
	ACE2-deficient mice	Ang II peptide was administered by i.v. infusion in wild-type and ACE2-deficient mice	Blood pressure measurements were substantially higher in the ACE2-deficient mice	Gurley *et al* [57]
	SHR and Wistar Kyoto (WKY) rats	Expression of ACE2 was examined in the kidney from SHR and normotensive WKY rats	The tubular expression of ACE2 fell while glomerular expression of ACE2 was paradoxically increased in the SHR	Tikellis *et al* [60]
	Sheep	Sheep were administered with betamethasone or vehicle at the 80th day of gestation and delivered at term	Antenatal steroid treatment resulted in the chronic alteration of ACE and ACE2 in the circulatory and tubular compartments of adolescent sheep, which may contribute to the higher blood pressure in this model of fetal programming–induced hypertension	Shaltout *et al* [61]

	Transgenic rats	Transgenic rats were generated in an SHRSP genetic background expressing human ACE2 in vascular smooth muscle cells by the use of the *SM22* promoter (SHRSP-ACE2 model)	Mean arterial blood pressure was reduced in SHRSP-ACE2, and the vasoconstrictive response to intraarterial administration of angiotensin II was attenuated	Rentzsch *et al* [62]
	SHR	Male WKY rats were randomized to receive either placebo or rhACE2 and were subsequently infused with Ang II	Treatment with rhACE2 partly corrected the hypertension, NADPH oxidase activation, and increased superoxide generation in the heart, kidney, and blood vessels	Lo *et al*[63]
	Mice	ACE2 activity was measured in kidney cortex from mice that had received injection of MLN-4760 or DX600	A marked increase in serum ACE2 activity. Mouse ACE2 abolished the hypertension induced by Ang II infusion. These effects were blocked by MLN-4760 but not by DX600	Ye *et al*[64]
Cardiovascular disease	ACE2-deficient mice	ACE2 mutant mice were generated, and heart parameters were measured	Genetic inactivation of ACE2 using homologous recombination resulted in increased AngII peptide levels, upregulation of hypoxia genes in the heart, and severe cardiac dysfunction	Crackower *et al*[56]
	ACE2-deficient mice	Ang II peptide was administered by i.v. infusion in WT and ACE2-deficient mice	No evidence for a role of ACE2 in the regulation of cardiac structure or function was found	Gurley *et al* [57]
	SD rats	Lentiviral vector encoding mouse ACE2 (lenti-mACE2) or GFP was injected intracardially in Sprague–Dawley rats	ACE2 overexpression resulted in protective effects on AngII-induced cardiac hypertrophy and fibrosis	Huentelman *et al*[71]
	SHR	Lentiviral vector encoding mouse ACE2 (lenti-mACE2) or GFP was injected intracardially in SHR and normotensive WKY rats	ACE2 overexpression exerted protective effects on high BP and cardiac pathophysiology induced by hypertension in the SHR	Díez-Freire *et al*[72]
	Rabbits	66 male New Zealand white rabbits were fed an atherogenic chow and were randomly divided into three groups: Treatment with a suspension of Ad-ACE2, treatment with a suspension of Ad-EGFP, and no treatment	ACE2 inhibited the development of early atherosclerotic lesions by suppressing the growth of VSMCs and improving endothelial function	Zhang *et al* [73]
Acute lung injury	ACE2 mutant mice	Acid aspiration-induced, sepsis-induced, and endotoxin-induced acute lung injury animal models were generated. Mice received intraperitoneal injections of rhACE2 protein	ACE2 and AT2 protected mice from severe acute lung injury. rhACE2 can protect mice from severe acute lung injury	Imai *et al*[82]
	ACE2 knockout mice	Mice were intranasally inoculated with SARS-CoV virus	SARS-CoV receptor ACE2 had a protective role in acute lung failure	Kuba *et al*[84]
	BALB/c mice	LPS-induced acute lung injury mice were treated with ACE2 activator resorcinolnaphthalein (RES) or ACE2 inhibitor MLN-4760	ACE2 activation can reduce the severity of LPS-induced acute lung injury *via* the AMPK/mTOR pathway	Zhang *et al* [87]
	c57BL/6J mice	Transgenic mice expressing the human ACE2 receptor driven by the cytokeratin-18 (K18) gene promoter (K18-hACE2)	Intranasal inoculation of SARS-CoV-2 in K18-hACE2 mice resulted in high levels of viral infection in lungs	Winkler *et al* [91]
	c57BL/6J mice	Mice expressing hACE2 in the lung were transduced by oropharyngeal delivery of the recombinant human adenovirus type 5 that expresses hACE2 (Ad5-hACE2)	Mice were infected with SARS-CoV-2 and developed interstitial pneumonia associated with perivascular inflammation, accompanied by a higher viral load in the lungs	Han *et al*[92]

UAE: Urinary albumin excretion; STZ: Streptozotocin; SD rats: Sprague-Dawley rats; SHR: Spontaneously hypertensive rats; SHRSP: Stroke-prone spontaneously hypertensive rats; SM22: Smooth muscle 22α; LPS: Lipopolysaccharide; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; ACE2: Angiotensin-converting enzyme 2.

hypertension. These authors generated transgenic rats in a SHRSP genetic background, called SHRSP-ACE2, which expressed human ACE2 in vascular smooth muscle cells under the control of the smooth muscle 22α promoter. They found that endothelial function was significantly improved in SHRSP-ACE2 rats compared with SHRSP rats. These data indicate that vascular ACE2 overexpression in SHRSP reduces hypertension, probably through local Ang II degradation and by improving endothelial function. Existing animal studies have shown that the administration of recombinant ACE2 (rACE2) degrades Ang II, lowers blood pressure, and attenuates Ang II–induced

Table 4 List of epidemiological studies on the role of angiotensin-converting enzyme 2 in diabetes, hypertension, cardiovascular disease, and acute lung injury

Disease	Source country	Patients	Main findings	Ref.
Diabetes	Canada	Renal biopsies from 13 diabetic and 8 control patients	ACE2 mRNA and protein expression were significantly reduced in both the glomeruli and proximal tubules of the diabetic patients	Reich *et al* [53]
	Japan	66 nondiabetic and 8 diabetic patients with biopsy-proven renal diseases	*ACE2* mRNA expression was not significantly changed in the diabetic patients	Konoshita *et al*[54]
	China	275 Uygur T2D patients and 272 nondiabetic Uygur individuals	*ACE2* SNPs rs1978124, rs2048683, rs2074192, rs233575, rs4240157, rs4646156, rs4646188, and rs879922 were associated with T2D	Liu e*t al* [55]
Hypertension	Australia	503 Caucasian subjects with type 2 diabetes	Genetic variation in *ACE2* was associated with hypertension and reduced systolic function in men, and hypertension and increased LV mass in women	Patel *et al* [65]
	China	275 Uygur T2D patients and 272 nondiabetic Uygur individuals	*ACE2* SNPs rs2048683, rs233575, rs4240157, rs4646156, rs4646188, and rs879922 were associated with increased SBP, while rs2074192, rs4646188, and rs879922 were associated elevated DBP	Liu *et al*[55]
	China	402 hypertensive patients and 233 normotensive individuals	*ACE2* variant rs2074192 was associated with EH, while rs4240157, rs4646155, and rs4830542 were associated with EH- and hypertension-related atrial fibrillation and left atrial remodeling	Luo *et al* [66]
	China	3408 untreated hypertensive patients	The T allele of *ACE2* rs2106809 was found to confer a 1.6-fold risk for hypertension in women	Fan *et al* [67]
	China	96 patients with EH and 96 healthy controls	Aberrant methylation of the *ACE2* promoter may be associated with EH risk	Fan *et al* [68]
Cardiovascular disease	United States	11 individuals with dilated cardiomyopathy, 15 individuals with hypertrophic cardiomyopathy, and 16 controls with nonfailing hearts from the Penn Human Heart Tissue Biobank	ACE2 expression was downregulated in fibroblasts, pericytes, and vascular smooth muscle but upregulated in cardiomyocytes in dilated cardiomyopathy and hypertrophic cardiomyopathy	Fan *et al* [67]
	Hungary	45 healthy individuals, 239 hypertensiveindividuals,141 patients with heart failure (HF) and reduced ejection fraction (HFrEF), and 47 patients with HF and preserved ejection fraction (HFpEF)	ACE2 activity was further increased in HFrEF patients. Serum ACE2 activity was negatively correlated with left ventricular systolic function in HFrEF	Úri *et al*[74]
	United States	113 patients with chronic systolic heart failure	Elevated plasma soluble ACE2 (sACE2) activity was associated with greater severity of myocardial dysfunction and was an independent predictor of adverse clinical events	Epelman *et al*[75]
	14 countries across five continents	10753 Prospective Urban Rural Epidemiology participants	Increased concentration of plasma ACE2 was associated with a higher risk of incident heart failure, myocardial infarction, stroke, and diabetes	Narula *et al* [76]
	Italy	Healthy subjects (C) and EH and Bartter's/Gitelman's (BS/GS) patients	ACE2 was significantly elevated in BS/GS compared with either C or EH	Calò*et al* [77]
	China	275 Uygur T2D patients and 272 nondiabetic Uygur individuals	*ACE2* SNPs rs2074192 and rs879922 were associated with carotid arteriosclerosis stenosis and *ACE2* SNPs rs2048683, rs4240157, rs4646156, rs4646188, and rs879922 were linked to heavier left heart remodeling	Liu *et al*[55]
Acute lung injury	United States	44 patients with acute respiratory distress syndrome (ARDS)	GSK2586881, a rhACE2, was well-tolerated in patients with ARDS, and has been found to reduce Ang II levels and increase Ang 1-7 levels	Khan *et al* [94]

T2D: type 2 diabetes; EH: Essential hypertension; ACE2: Angiotensin-converting enzyme 2; ARDS: Acute respiratory distress syndrome; SNP: Single nucleotide polymorphism; HF: Heart failure; HFrEF: Heart failure and reduced ejection fraction; HFpEF: Heart failure and preserved ejection fraction; Ang 1-7: Angiotensin 1-7.

organ injury. One study showed that in the SHR model, rhACE2 partly corrected hypertension and NADPH oxidase activation and increased superoxide generation in

the heart, kidney, and blood vessels over a 14-d period[63]. Moreover, the prevention of Ang II-induced hypertension by mouse rACE2 was completely abolished by the specific ACE2 inhibitor MLN-4760, a nonpeptide inhibitor no longer available from Millennium Pharmaceuticals[64].

In human studies, Patel *et al*[65] found that in Caucasians with T2D, genetic variation in *ACE2* is associated with hypertension and reduced systolic function in men and hypertension and increased left ventricular mass in women. Liu *et al*[55] found that the *ACE2* SNPs rs2048683, rs233575, rs4240157, rs4646156, rs4646188, and rs879922 were associated with increased systolic blood pressure (SBP), while rs2074192, rs4646188, and rs879922 were associated with elevated diastolic blood pressure in Uygur T2D patients. Luo *et al*[66] revealed that the *ACE2* variant rs2074192 was associated with essential hypertension (EH), while three *ACE2* variants (rs4240157, rs4646155, and rs4830542) were associated with EH- and hypertension-related atrial fibrillation and left atrial remodeling in south Xinjiang, China. In another study, the *ACE2* rs2106809 T allele was found to confer a 1.6-fold risk for hypertension in women[67]. Additionally, one study showed that aberrant methylation of the *ACE2* promoter may be associated with EH risk[68].

These findings indicate that ACE2 is a key regulator that maintains the balance of blood pressure. In animal studies, it has been demonstrated that the administration of recombinant ACE2 has a beneficial effect on the treatment of hypertension. Currently, large clinical trials to explore this and related alternative interventions are underway.

ACE2 and cardiac function, ventricular remodeling, and HF

SARS-CoV-2 enters the upper respiratory epithelium and lungs predominantly through ACE2. Nevertheless, in a single-center report of 416 patients hospitalized with COVID-19, 19.7% showed evidence of cardiac injury, suggesting a possible pathologic role for myocardial ACE2 expression[69]. ACE2 is present on endothelial cells and can undergo so-called shedding into the circulation. In patients with cardiovascular disease, increased ACE2 activity in the circulation predicts adverse cardiovascular outcomes in patients with HF, coronary artery disease, and aortic stenosis[70].

Crackower *et al*[56] demonstrated the first evidence that ACE2 may have a role in cardiac function. They found that ACE2 deletion in mice resulted in a severe heart contractility defect, increased levels of Ang II in the kidney, heart, and plasma, and upregulation of hypoxia-induced genes in the heart. Conversely, Gurley *et al*[57] reported that ACE2 deletion enhanced susceptibility to Ang II-induced hypertension but had no effect on cardiac structure or function. Huentelman *et al*[71] showed that ACE2 overexpression protects the heart from Ang II-induced hypertrophy and fibrosis. Another study on SHR hypertensive rats also showed that ACE2 overexpression exerted protective effects against high blood pressure and cardiac pathophysiology induced by hypertension[72]. Similarly, in a rabbit atherosclerosis model, local overexpression of ACE2 significantly inhibited the development of early atherosclerotic lesions[73].

In humans, increased circulating ACE2 activity is associated with coronary heart disease and HF, and a large proportion of the variation in plasma ACE2 levels is attributed to hereditary factors. One study showed that ACE2 activity was significantly increased in HF patients with reduced ejection fraction (HFrEF). Serum ACE2 activity was negatively correlated with left ventricular systolic function in HfrEF[74]. In addition, one study measured soluble ACE2 (sACE2) activity in 113 patients with chronic systolic HF and showed that elevated plasma sACE2 activity was associated with greater severity of myocardial dysfunction, which indicated that plasma sACE2 activity might be an independent predictor of adverse clinical events[75]. A recent study published in Lancet presented one of the largest epidemiological datasets on plasma ACE2 concentration in the general population[76]. They performed a case-cohort study involving 10753 participants from the multinational Prospective Urban Rural Epidemiology study, including 5084 patients randomly selected as the subcohort and 5669 with an incident event of interest. They reported that ACE2 concentration was the highest-ranked independent predictor of death compared with standard cardiovascular risk markers (smoking, diabetes, SBP, non-high density lipoprotein cholesterol, and body mass index). An increased concentration of plasma ACE2 was associated with an increased risk of all-cause mortality [hazard ratio (HR): 1.35 per 1 standard deviation (SD) increase; 95% confidence interval (CI): 1.29-1.43], incident HF (HR: 1.27 per 1 SD increase; 95%CI: 1.10-1.46), stroke (HR: 1.21 per 1 SD increase; 95%CI: 1.10-1.32), myocardial infarction (HR: 1.23 per 1 SD increase; 95%CI: 1.13-1.33), and incident diabetes (HR: 1.44 per 1 SD increase; 95%CI: 1.36-1.52). Other studies have investigated whether the *ACE2* gene is associated with left ventricular hypertrophy and coronary artery disease. The *ACE2* SNPs most frequently used in

association studies are rs2285666 and rs1978124[77]. Recently, Liu *et al*[55] found that the *ACE2* SNPs rs2074192 and rs879922 were associated with carotid arteriosclerosis stenosis and that the *ACE2* SNPs rs2048683, rs4240157, rs4646156, rs4646188, and rs879922 were linked to more substantial left heart remodeling. Furthermore, one study assessed ACE2 expression by performing bulk and single nucleus RNA-Seq on the left ventricles of 11 individuals with dilated cardiomyopathy, 15 individuals with hypertrophic cardiomyopathy, and 16 controls with nonfailing hearts from the Penn Human Heart Tissue Biobank. They found that cardiac ACE2 expression was down-regulated in fibroblasts, pericytes, and vascular smooth muscle but upregulated in cardiomyocytes[69].

Investigations of ACE2 as well as its role in cardiac function and HF will undoubtedly provide greater insight into the roles of this enzyme. However, carefully conducted large-scale clinical studies are urgently needed to clarify the potential role of ACE2 in cardiovascular diseases more precisely.

ACE2 and acute lung injury

Like many other organ lung cells also have a local RAS[78], which influences the pathogenesis of lung injury *via* cellular effects, including changes in vascular permeability, vascular tone, fibroblast activity, or alveolar epithelial cell apoptosis[79, 80]. ACE2 plays a pivotal role in Ang II degradation in the RAS cascade and thus limits inflammation and fibrosis in the lung[81]. Imai *et al*[82] investigated the role of ACE2 in acute respiratory distress syndrome (ARDS) by using ACE2 knockout mice. In three different ARDS models (acid-aspiration-induced, endotoxin-induced, and peritoneal sepsis-induced ARDS), it was shown that a loss of ACE2 expression in mutant mice resulted in enhanced vascular permeability, increased lung edema, engendered neutrophil accumulation, and worsened lung function. Importantly, treatment with catalytically active recombinant ACE2 protein improved the symptoms of acute lung injury in both wild-type mice and ACE2 knockout mice. Thus, ACE2 plays a protective role in acute lung injury. Mechanically, the finding that reduced ACE2 on lung cell surfaces is correlated with lung damage due to an uncontrolled RAS cascade is supported by data about the effects of long-lasting hyperoxia on pulmonary tissue[82-86]. In addition, a study revealed that ACE2 activation can reduce the severity of lipopolysaccharide-induced acute lung injury *via* the activated serine/threonine protein kinase/mammalian target of rapamycin pathway[87].

In the current pandemic of COVID-19, both bioinformatics modeling and *in vitro* experiments indicate that SARS-CoV-2 likely utilizes ACE2 as a receptor to gain entry into human cells[88-90]. A recent study evaluated lung function in a mouse model of SARS-CoV-2 infection. They used transgenic mice expressing the human ACE2 (hACE2) receptor driven by the cytokeratin-18 (*K18*) gene promoter (K18-hACE2) and found that intranasal inoculation of SARS-CoV-2 in K18-hACE2 mice resulted in high levels of viral infection in the lungs, with spread to other organs[91]. Similarly, one study also used a model of mice expressing hACE2 in the lung. In this study, the mice were transduced by oropharyngeal delivery of recombinant human adenovirus type 5 expressing hACE2[92]. They found that mice were infected with SARS-CoV-2 at day 4 post-transduction and developed interstitial pneumonia related to perivascular inflammation. On the other hand, as described above, a similar decrease in ACE2 has also been seen in cases of COVID-19 with severe lung injury, which might be attributable to the negative consequences that arise from insufficient Ang II degradation[93]. In human trials, GSK2586881, a rhACE2, was well tolerated in 44 patients with ARDS and has been found to reduce Ang II levels and increase Ang 1-7 levels, although it failed to improve the physiological and clinical indicators of ARDS in patients[94]. This study likely represents the first clinical application of rhACE2 in the field of ARDS, so we speculate that rhACE2 may become one of the most promising approaches for protecting against lung injury in patients with COVID-19.

Based on the above description, ACE2 plays a complex role in COVID-19-induced acute lung injury. On the one hand, high levels of ACE2 receptors on the cell surface may accelerate the invasion of SARS-CoV-2 during the very early phase of infection. On the other hand, low levels of ACE2 can ultimately worsen the disease course due to insufficient Ang II conversion in cases of severe COVID-19 with pulmonary complications.

CONCLUSION

As a vital component of the RAS, ACE2 is closely related to the occurrence and

development of RAS-associated diseases. A better understanding of the biological functions of ACE2 will be beneficial to the treatment. Previous studies show that a high-salt diet can decrease the expression of ACE2 and cause RAS disorders. Recent reports have not yet indicated that ARBs or ACEIs will increase the level of ACE2, thereby aggravating SARS-CoV-2 infection. Therefore, the mechanisms involved need to be further improved. As ACE2 is an important receptor through which SARS-CoV-2 can invade cells, further studies on ACE2 should focus on the development of drugs that inhibit the virus from entering cells and impede the binding of the S protein to ACE2 for COVID-19 treatment. More research on ACE2 should be conducted in the future to carry out targeted and effective treatment at a higher level.

REFERENCES

1 **Li W**, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003; **426**: 450-454 [PMID: 14647384 DOI: 10.1038/nature02145]

2 **Fang L**, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med* 2020; **8**: e21 [PMID: 32171062 DOI: 10.1016/S2213-2600(20)30116-8]

3 **Zheng YY**, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol* 2020; **17**: 259-260 [PMID: 32139904 DOI: 10.1038/s41569-020-0360-5]

4 **Yan R**, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020; **367**: 1444-1448 [PMID: 32132184 DOI: 10.1126/science.abb2762]

5 **Tipnis SR**, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000; **275**: 33238-33243 [PMID: 10924499 DOI: 10.1074/jbc.M002615200]

6 **Donoghue M**, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000; **87**: E1-E9 [PMID: 10969042 DOI: 10.1161/01.res.87.5.e1]

7 **Castardeli C**, Sartório CL, Pimentel EB, Forechi L, Mill JG. The ACE 2 activator diminazene aceturate (DIZE) improves left ventricular diastolic dysfunction following myocardial infarction in rats. *Biomed Pharmacother* 2018; **107**: 212-218 [PMID: 30092400 DOI: 10.1016/j.biopha.2018.07.170]

8 **Bradford CN**, Ely DR, Raizada MK. Targeting the vasoprotective axis of the renin-angiotensin system: a novel strategic approach to pulmonary hypertensive therapy. *Curr Hypertens Rep* 2010; **12**: 212-219 [PMID: 20556668 DOI: 10.1007/s11906-010-0122-6]

9 **Turner AJ**, Hooper NM. The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends Pharmacol Sci* 2002; **23**: 177-183 [PMID: 11931993 DOI: 10.1016/s0165-6147(00)01994-5]

10 **Turner AJ**, Hiscox JA, Hooper NM. ACE2: from vasopeptidase to SARS virus receptor. *Trends Pharmacol Sci* 2004; **25**: 291-294 [PMID: 15165741 DOI: 10.1016/j.tips.2004.04.001]

11 **Passos-Silva DG**, Verano-Braga T, Santos RA. Angiotensin-(1-7): beyond the cardio-renal actions. *Clin Sci (Lond)* 2013; **124**: 443-456 [PMID: 23249272 DOI: 10.1042/CS20120461]

12 **Zhang YH**, Zhang YH, Dong XF, Hao QQ, Zhou XM, Yu QT, Li SY, Chen X, Tengbeh AF, Dong B, Zhang Y. ACE2 and Ang-(1-7) protect endothelial cell function and prevent early atherosclerosis by inhibiting inflammatory response. *Inflamm Res* 2015; **64**: 253-260 [PMID: 25721616 DOI: 10.1007/s00011-015-0805-1]

13 **Lovren F**, Pan Y, Quan A, Teoh H, Wang G, Shukla PC, Levitt KS, Oudit GY, Al-Omran M, Stewart DJ, Slutsky AS, Peterson MD, Backx PH, Penninger JM, Verma S. Angiotensin converting enzyme-2 confers endothelial protection and attenuates atherosclerosis. *Am J Physiol Heart Circ Physiol* 2008; **295**: H1377-H1384 [PMID: 18660448 DOI: 10.1152/ajpheart.00331.2008]

14 **Wang Y**, Tikellis C, Thomas MC, Golledge J. Angiotensin converting enzyme 2 and atherosclerosis. *Atherosclerosis* 2013; **226**: 3-8 [PMID: 22947420 DOI: 10.1016/j.atherosclerosis.2012.08.018]

15 **Fraga-Silva RA**, Da Silva DG, Montecucco F, Mach F, Stergiopulos N, da Silva RF, Santos RA. The angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas receptor axis: a potential target for treating thrombotic diseases. *Thromb Haemost* 2012; **108**: 1089-1096 [PMID: 23093373 DOI: 10.1160/TH12-06-0396]

16 **Lubel JS**, Herath CB, Tchongue J, Grace J, Jia Z, Spencer K, Casley D, Crowley P, Sievert W, Burrell LM, Angus PW. Angiotensin-(1-7), an alternative metabolite of the renin-angiotensin system, is up-regulated in human liver disease and has antifibrotic activity in the bile-duct-ligated rat. *Clin Sci (Lond)* 2009; **117**: 375-386 [PMID: 19371232 DOI: 10.1042/CS20080647]

17 **Kuba K**, Imai Y, Penninger JM. Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. *Circ J* 2013; **77**: 301-308 [PMID: 23328447 DOI: 10.1253/circj.cj-12-1544]

18 **Berger RC**, Vassallo PF, Crajoinas Rde O, Oliveira ML, Martins FL, Nogueira BV, Motta-Santos D,

Araújo IB, Forechi L, Girardi AC, Santos RA, Mill JG. Renal Effects and Underlying Molecular Mechanisms of Long-Term Salt Content Diets in Spontaneously Hypertensive Rats. *PLoS One* 2015; **10**: e0141288 [PMID: 26495970 DOI: 10.1371/journal.pone.0141288]

19 **Samuel P**, Ali Q, Sabuhi R, Wu Y, Hussain T. High Na intake increases renal angiotensin II levels and reduces expression of the ACE2-AT(2)R-MasR axis in obese Zucker rats. *Am J Physiol Renal Physiol* 2012; **303**: F412-F419 [PMID: 22592638 DOI: 10.1152/ajprenal.00097.2012]

20 **Bernardi S**, Toffoli B, Zennaro C, Tikellis C, Monticone S, Losurdo P, Bellini G, Thomas MC, Fallo F, Veglio F, Johnston CI, Fabris B. High-salt diet increases glomerular ACE/ACE2 ratio leading to oxidative stress and kidney damage. *Nephrol Dial Transplant* 2012; **27**: 1793-1800 [PMID: 22036945 DOI: 10.1093/ndt/gfr600]

21 **Shimoura CG**, Lincevicius GS, Nishi EE, Girardi AC, Simon KA, Bergamaschi CT, Campos RR. Increased Dietary Salt Changes Baroreceptor Sensitivity and Intrarenal Renin-Angiotensin System in Goldblatt Hypertension. *Am J Hypertens* 2017; **30**: 28-36 [PMID: 27629265 DOI: 10.1093/ajh/hpw107]

22 **Varagic J**, Ahmad S, Brosnihan KB, Groban L, Chappell MC, Tallant EA, Gallagher PE, Ferrario CM. Decreased cardiac Ang-(1-7) is associated with salt-induced cardiac remodeling and dysfunction. *Ther Adv Cardiovasc Dis* 2010; **4**: 17-25 [PMID: 19946038 DOI: 10.1177/1753944709353337]

23 **Takeda Y**, Zhu A, Yoneda T, Usukura M, Takata H, Yamagishi M. Effects of aldosterone and angiotensin II receptor blockade on cardiac angiotensinogen and angiotensin-converting enzyme 2 expression in Dahl salt-sensitive hypertensive rats. *Am J Hypertens* 2007; **20**: 1119-1124 [PMID: 17903697 DOI: 10.1016/j.amjhyper.2007.05.008]

24 **Cao G**, Della Penna SL, Kouyoumdzian NM, Choi MR, Gorzalczany S, Fernández BE, Toblli JE, Rosón MI. Immunohistochemical expression of intrarenal renin angiotensin system components in response to tempol in rats fed a high salt diet. *World J Nephrol* 2017; **6**: 29-40 [PMID: 28101449 DOI: 10.5527/wjn.v6.i1.29]

25 **Tikellis C**, Bernardi S, Burns WC. Angiotensin-converting enzyme 2 is a key modulator of the renin-angiotensin system in cardiovascular and renal disease. *Curr Opin Nephrol Hypertens* 2011; **20**: 62-68 [PMID: 21099686 DOI: 10.1097/MNH.0b013e328341164a]

26 **Ferrario CM**, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 2005; **111**: 2605-2610 [PMID: 15897343 DOI: 10.1161/CIRCULATIONAHA.104.510461]

27 **Ocaranza MP**, Godoy I, Jalil JE, Varas M, Collantes P, Pinto M, Roman M, Ramirez C, Copaja M, Diaz-Araya G, Castro P, Lavandero S. Enalapril attenuates downregulation of Angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension* 2006; **48**: 572-578 [PMID: 16908757 DOI: 10.1161/01.HYP.0000237862.94083.45]

28 **Ishiyama Y**, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension* 2004; **43**: 970-976 [PMID: 15007027 DOI: 10.1161/01.HYP.0000124667.34652.1a]

29 **Soler MJ**, Ye M, Wysocki J, William J, Lloveras J, Batlle D. Localization of ACE2 in the renal vasculature: amplification by angiotensin II type 1 receptor blockade using telmisartan. *Am J Physiol Renal Physiol* 2009; **296**: F398-F405 [PMID: 19004932 DOI: 10.1152/ajprenal.90488.2008]

30 **Burrell LM**, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, Tikellis C, Grant SL, Lew RA, Smith AI, Cooper ME, Johnston CI. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J* 2005; **26**: 369-75; discussion 322 [PMID: 15671045 DOI: 10.1093/eurheartj/ehi114]

31 **Burchill LJ**, Velkoska E, Dean RG, Griggs K, Patel SK, Burrell LM. Combination renin-angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions. *Clin Sci (Lond)* 2012; **123**: 649-658 [PMID: 22715807 DOI: 10.1042/CS20120162]

32 **Sriram K**, Insel PA. Risks of ACE Inhibitor and ARB Usage in COVID-19: Evaluating the Evidence. *Clin Pharmacol Ther* 2020; **108**: 236-241 [PMID: 32320478 DOI: 10.1002/cpt.1863]

33 **Li J**, Wang X, Chen J, Zhang H, Deng A. Association of Renin-Angiotensin System Inhibitors With Severity or Risk of Death in Patients With Hypertension Hospitalized for Coronavirus Disease 2019 (COVID-19) Infection in Wuhan, China. *JAMA Cardiol* 2020; **5**: 825-830 [PMID: 32324209 DOI: 10.1001/jamacardio.2020.1624]

34 **Chu C**, Zeng S, Hasan AA, Hocher CF, Krämer BK, Hocher B. Comparison of infection risks and clinical outcomes in patients with and without SARS-CoV-2 lung infection under renin-angiotensin-aldosterone system blockade: Systematic review and meta-analysis. *Br J Clin Pharmacol* 2020 [PMID: 33217033 DOI: 10.1111/bcp.14660]

35 **Li XC**, Zhang J, Zhuo JL. The vasoprotective axes of the renin-angiotensin system: Physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacol Res* 2017; **125**: 21-38 [PMID: 28619367 DOI: 10.1016/j.phrs.2017.06.005]

36 **Sommerstein R**, Kochen MM, Messerli FH, Gräni C. Coronavirus Disease 2019 (COVID-19): Do Angiotensin-Converting Enzyme Inhibitors/Angiotensin Receptor Blockers Have a Biphasic Effect? *J Am Heart Assoc* 2020; **9**: e016509 [PMID: 32233753 DOI: 10.1161/JAHA.120.016509]

37 **Guo J**, Huang Z, Lin L, Lv J. Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease: A Viewpoint on the Potential Influence of Angiotensin-Converting Enzyme Inhibitors/Angiotensin Receptor Blockers on Onset and Severity of Severe Acute Respiratory Syndrome Coronavirus 2

Infection. *J Am Heart Assoc* 2020; **9**: e016219 [PMID: 32233755 DOI: 10.1161/JAHA.120.016219]
38 **Alexandre J**, Cracowski JL, Richard V, Bouhanick B; 'Drugs, COVID-19' working group of the French Society of Pharmacology, Therapeutics. Renin-angiotensin-aldosterone system and COVID-19 infection. *Ann Endocrinol (Paris)* 2020; **81**: 63-67 [PMID: 32370986 DOI: 10.1016/j.ando.2020.04.005]
39 **Warner FJ**, Guy JL, Lambert DW, Hooper NM, Turner AJ. Angiotensin Converting Enzyme-2 (ACE2) and its Possible Roles in Hypertension, Diabetes and Cardiac Function. *Lett Pept Sci* 2003; **10**: 377-385 [PMID: 32214681 DOI: 10.1007/s10989-004-2387-6]
40 **Mizuiri S**, Ohashi Y. ACE and ACE2 in kidney disease. *World J Nephrol* 2015; **4**: 74-82 [PMID: 25664248 DOI: 10.5527/wjn.v4.i1.74]
41 **Batlle D**, Jose Soler M, Ye M. ACE2 and diabetes: ACE of ACEs? *Diabetes* 2010; **59**: 2994-2996 [PMID: 21115782 DOI: 10.2337/db10-1205]
42 **Bindom SM**, Lazartigues E. The sweeter side of ACE2: physiological evidence for a role in diabetes. *Mol Cell Endocrinol* 2009; **302**: 193-202 [PMID: 18948167 DOI: 10.1016/j.mce.2008.09.020]
43 **Rao S**, Lau A, So HC. Exploring Diseases/Traits and Blood Proteins Causally Related to Expression of ACE2, the Putative Receptor of SARS-CoV-2: A Mendelian Randomization Analysis Highlights Tentative Relevance of Diabetes-Related Traits. *Diabetes Care* 2020; **43**: 1416-1426 [PMID: 32430459 DOI: 10.2337/dc20-0643]
44 **Goru SK**, Kadakol A, Malek V, Pandey A, Sharma N, Gaikwad AB. Diminazene aceturate prevents nephropathy by increasing glomerular ACE2 and AT_2 receptor expression in a rat model of type1 diabetes. *Br J Pharmacol* 2017; **174**: 3118-3130 [PMID: 28688122 DOI: 10.1111/bph.13946]
45 **Chodavarapu H**, Grobe N, Somineni HK, Salem ES, Madhu M, Elased KM. Rosiglitazone treatment of type 2 diabetic db/db mice attenuates urinary albumin and angiotensin converting enzyme 2 excretion. *PLoS One* 2013; **8**: e62833 [PMID: 23646149 DOI: 10.1371/journal.pone.0062833]
46 **Ye M**, Wysocki J, William J, Soler MJ, Cokic I, Batlle D. Glomerular localization and expression of Angiotensin-converting enzyme 2 and Angiotensin-converting enzyme: implications for albuminuria in diabetes. *J Am Soc Nephrol* 2006; **17**: 3067-3075 [PMID: 17021266 DOI: 10.1681/ASN.2006050423]
47 **Tikellis C**, Johnston CI, Forbes JM, Burns WC, Burrell LM, Risvanis J, Cooper ME. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension* 2003; **41**: 392-397 [PMID: 12623933 DOI: 10.1161/01.HYP.0000060689.38912.CB]
48 **Ye M**, Wysocki J, Naaz P, Salabat MR, LaPointe MS, Batlle D. Increased ACE 2 and decreased ACE protein in renal tubules from diabetic mice: a renoprotective combination? *Hypertension* 2004; **43**: 1120-1125 [PMID: 15078862 DOI: 10.1161/01.HYP.0000126192.27644.76]
49 **Wong DW**, Oudit GY, Reich H, Kassiri Z, Zhou J, Liu QC, Backx PH, Penninger JM, Herzenberg AM, Scholey JW. Loss of angiotensin-converting enzyme-2 (Ace2) accelerates diabetic kidney injury. *Am J Pathol* 2007; **171**: 438-451 [PMID: 17600118 DOI: 10.2353/ajpath.2007.060977]
50 **Tikellis C**, Bialkowski K, Pete J, Sheehy K, Su Q, Johnston C, Cooper ME, Thomas MC. ACE2 deficiency modifies renoprotection afforded by ACE inhibition in experimental diabetes. *Diabetes* 2008; **57**: 1018-1025 [PMID: 18235039 DOI: 10.2337/db07-1212]
51 **Oudit GY**, Liu GC, Zhong J, Basu R, Chow FL, Zhou J, Loibner H, Janzek E, Schuster M, Penninger JM, Herzenberg AM, Kassiri Z, Scholey JW. Human recombinant ACE2 reduces the progression of diabetic nephropathy. *Diabetes* 2010; **59**: 529-538 [PMID: 19934006 DOI: 10.2337/db09-1218]
52 **Liu CX**, Hu Q, Wang Y, Zhang W, Ma ZY, Feng JB, Wang R, Wang XP, Dong B, Gao F, Zhang MX, Zhang Y. Angiotensin-converting enzyme (ACE) 2 overexpression ameliorates glomerular injury in a rat model of diabetic nephropathy: a comparison with ACE inhibition. *Mol Med* 2011; **17**: 59-69 [PMID: 20844835 DOI: 10.2119/molmed.2010.00111]
53 **Reich HN**, Oudit GY, Penninger JM, Scholey JW, Herzenberg AM. Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. *Kidney Int* 2008; **74**: 1610-1616 [PMID: 19034303 DOI: 10.1038/ki.2008.497]
54 **Konoshita T**, Wakahara S, Mizuno S, Motomura M, Aoyama C, Makino Y, Kawai Y, Kato N, Koni I, Miyamori I, Mabuchi H. Tissue gene expression of renin-angiotensin system in human type 2 diabetic nephropathy. *Diabetes Care* 2006; **29**: 848-852 [PMID: 16567826 DOI: 10.2337/diacare.29.04.06.dc05-1873]
55 **Liu C**, Li Y, Guan T, Lai Y, Shen Y, Zeyaweiding A, Zhao H, Li F, Maimaiti T. ACE2 polymorphisms associated with cardiovascular risk in Uygurs with type 2 diabetes mellitus. *Cardiovasc Diabetol* 2018; **17**: 127 [PMID: 30227878 DOI: 10.1186/s12933-018-0771-3]
56 **Crackower MA**, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, da Costa J, Zhang L, Pei Y, Scholey J, Ferrario CM, Manoukian AS, Chappell MC, Backx PH, Yagil Y, Penninger JM. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 2002; **417**: 822-828 [PMID: 12075344 DOI: 10.1038/nature00786]
57 **Gurley SB**, Allred A, Le TH, Griffiths R, Mao L, Philip N, Haystead TA, Donoghue M, Breitbart RE, Acton SL, Rockman HA, Coffman TM. Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. *J Clin Invest* 2006; **116**: 2218-2225 [PMID: 16878172 DOI: 10.1172/JCI16980]
58 **Chappell MC**. Does ACE2 contribute to the development of hypertension? *Hypertens Res* 2010; **33**: 107-109 [PMID: 20019704 DOI: 10.1038/hr.2009.207]
59 **Yamamoto K**, Ohishi M, Katsuya T, Ito N, Ikushima M, Kaibe M, Tatara Y, Shiota A, Sugano S, Takeda S, Rakugi H, Ogihara T. Deletion of angiotensin-converting enzyme 2 accelerates pressure

overload-induced cardiac dysfunction by increasing local angiotensin II. *Hypertension* 2006; **47**: 718-726 [PMID: 16505206 DOI: 10.1161/01.HYP.0000205833.89478.5b]

60 **Tikellis C**, Cooper ME, Bialkowski K, Johnston CI, Burns WC, Lew RA, Smith AI, Thomas MC. Developmental expression of ACE2 in the SHR kidney: a role in hypertension? *Kidney Int* 2006; **70**: 34-41 [PMID: 16710353 DOI: 10.1038/sj.ki.5000428]

61 **Shaltout HA**, Figueroa JP, Rose JC, Diz DI, Chappell MC. Alterations in circulatory and renal angiotensin-converting enzyme and angiotensin-converting enzyme 2 in fetal programmed hypertension. *Hypertension* 2009; **53**: 404-408 [PMID: 19047579 DOI: 10.1161/HYPERTENSIONAHA.108.124339]

62 **Rentzsch B**, Todiras M, Iliescu R, Popova E, Campos LA, Oliveira ML, Baltatu OC, Santos RA, Bader M. Transgenic angiotensin-converting enzyme 2 overexpression in vessels of SHRSP rats reduces blood pressure and improves endothelial function. *Hypertension* 2008; **52**: 967-973 [PMID: 18809792 DOI: 10.1161/HYPERTENSIONAHA.108.114322]

63 **Lo J**, Patel VB, Wang Z, Levasseur J, Kaufman S, Penninger JM, Oudit GY. Angiotensin-converting enzyme 2 antagonizes angiotensin II-induced pressor response and NADPH oxidase activation in Wistar-Kyoto rats and spontaneously hypertensive rats. *Exp Physiol* 2013; **98**: 109-122 [PMID: 22750422 DOI: 10.1113/expphysiol.2012.067165]

64 **Ye M**, Wysocki J, Gonzalez-Pacheco FR, Salem M, Evora K, Garcia-Halpin L, Poglitsch M, Schuster M, Batlle D. Murine recombinant angiotensin-converting enzyme 2: effect on angiotensin II-dependent hypertension and distinctive angiotensin-converting enzyme 2 inhibitor characteristics on rodent and human angiotensin-converting enzyme 2. *Hypertension* 2012; **60**: 730-740 [PMID: 22777933 DOI: 10.1161/HYPERTENSIONAHA.112.198622]

65 **Patel SK**, Wai B, Ord M, MacIsaac RJ, Grant S, Velkoska E, Panagiotopoulos S, Jerums G, Srivastava PM, Burrell LM. Association of ACE2 genetic variants with blood pressure, left ventricular mass, and cardiac function in Caucasians with type 2 diabetes. *Am J Hypertens* 2012; **25**: 216-222 [PMID: 21993363 DOI: 10.1038/ajh.2011.188]

66 **Luo Y**, Liu C, Guan T, Li Y, Lai Y, Li F, Zhao H, Maimaiti T, Zeyaweiding A. Association of ACE2 genetic polymorphisms with hypertension-related target organ damages in south Xinjiang. *Hypertens Res* 2019; **42**: 681-689 [PMID: 30542083 DOI: 10.1038/s41440-018-0166-6]

67 **Fan X**, Wang Y, Sun K, Zhang W, Yang X, Wang S, Zhen Y, Wang J, Li W, Han Y, Liu T, Wang X, Chen J, Wu H, Hui R; Study Group for Pharmacogenomic Based Antihypertensive Drugs Selection, Effects and Side Effects, in Rural Area Chinese. Polymorphisms of ACE2 gene are associated with essential hypertension and antihypertensive effects of Captopril in women. *Clin Pharmacol Ther* 2007; **82**: 187-196 [PMID: 17473847 DOI: 10.1038/sj.clpt.6100214]

68 **Fan R**, Mao SQ, Gu TL, Zhong FD, Gong ML, Hao LM, Yin FY, Dong CZ, Zhang LN. Preliminary analysis of the association between methylation of the ACE2 promoter and essential hypertension. *Mol Med Rep* 2017; **15**: 3905-3911 [PMID: 28440441 DOI: 10.3892/mmr.2017.6460]

69 **Tucker NR**, Chaffin M, Bedi KC Jr, Papangeli I, Akkad AD, Arduini A, Hayat S, Eraslan G, Muus C, Bhattacharyya RP, Stegmann CM; Human Cell Atlas Lung Biological Network, Margulies KB, Ellinor PT; Human Cell Atlas Lung Biological Network Consortium Members. Myocyte-Specific Upregulation of *ACE2* in Cardiovascular Disease: Implications for SARS-CoV-2-Mediated Myocarditis. *Circulation* 2020; **142**: 708-710 [PMID: 32795091 DOI: 10.1161/CIRCULATIONAHA.120.047911]

70 **Ramchand J**, Burrell LM. Circulating ACE2: a novel biomarker of cardiovascular risk. *Lancet* 2020; **396**: 937-939 [PMID: 33010824 DOI: 10.1016/S0140-6736(20)32011-0]

71 **Huentelman MJ**, Grobe JL, Vazquez J, Stewart JM, Mecca AP, Katovich MJ, Ferrario CM, Raizada MK. Protection from angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats. *Exp Physiol* 2005; **90**: 783-790 [PMID: 16049057 DOI: 10.1113/expphysiol.2005.031096]

72 **Díez-Freire C**, Vázquez J, Correa de Adjounian MF, Ferrari MF, Yuan L, Silver X, Torres R, Raizada MK. ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR. *Physiol Genomics* 2006; **27**: 12-19 [PMID: 16788004 DOI: 10.1152/physiolgenomics.00312.2005]

73 **Zhang C**, Zhao YX, Zhang YH, Zhu L, Deng BP, Zhou ZL, Li SY, Lu XT, Song LL, Lei XM, Tang WB, Wang N, Pan CM, Song HD, Liu CX, Dong B, Zhang Y, Cao Y. Angiotensin-converting enzyme 2 attenuates atherosclerotic lesions by targeting vascular cells. *Proc Natl Acad Sci U S A* 2010; **107**: 15886-15891 [PMID: 20798044 DOI: 10.1073/pnas.1001253107]

74 **Úri K**, Fagyas M, Kertész A, Borbély A, Jenei C, Bene O, Csanádi Z, Paulus WJ, Édes I, Papp Z, Tóth A, Lizanecz E. Circulating ACE2 activity correlates with cardiovascular disease development. *J Renin Angiotensin Aldosterone Syst* 2016; **17** [PMID: 27965422 DOI: 10.1177/1470320316668435]

75 **Epelman S**, Shrestha K, Troughton RW, Francis GS, Sen S, Klein AL, Tang WH. Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes. *J Card Fail* 2009; **15**: 565-571 [PMID: 19700132 DOI: 10.1016/j.cardfail.2009.01.014]

76 **Narula S**, Yusuf S, Chong M, Ramasundarahettige C, Rangarajan S, Bangdiwala SI, van Eikels M, Leineweber K, Wu A, Pigeyre M, Paré G. Plasma ACE2 and risk of death or cardiometabolic diseases: a case-cohort analysis. *Lancet* 2020; **396**: 968-976 [PMID: 33010842 DOI: 10.1016/S0140-6736(20)31964-4]

77 **Calò LA**, Schiavo S, Davis PA, Pagnin E, Mormino P, D'Angelo A, Pessina AC. ACE2 and

angiotensin 1-7 are increased in a human model of cardiovascular hyporeactivity: pathophysiological implications. *J Nephrol* 2010; **23**: 472-477 [PMID: 20349406 DOI: 10.1089/end.2010.0121]

78 **Iwai M**, Horiuchi M. Devil and angel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2-angiotensin-(1-7)-Mas receptor axis. *Hypertens Res* 2009; **32**: 533-536 [PMID: 19461648 DOI: 10.1038/hr.2009.74]

79 **Tan WSD**, Liao W, Zhou S, Mei D, Wong WF. Targeting the renin-angiotensin system as novel therapeutic strategy for pulmonary diseases. *Curr Opin Pharmacol* 2018; **40**: 9-17 [PMID: 29288933 DOI: 10.1016/j.coph.2017.12.002]

80 **Morrell NW**, Stenmark KR. The renin-angiotensin system in pulmonary hypertension. *Am J Respir Crit Care Med* 2013; **187**: 1138-1139 [PMID: 23675718 DOI: 10.1164/rccm.201210-1872LE]

81 **Zhang PX**, Han CH, Zhou FJ, Li L, Zhang HM, Liu WW. Renin-angiotensin system and its role in hyperoxic acute lung injury. *Undersea Hyperb Med* 2016; **43**: 239-246 [PMID: 27416692]

82 **Imai Y**, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005; **436**: 112-116 [PMID: 16001071 DOI: 10.1038/nature03712]

83 **Sturrock BR**, Milne KM, Chevassut TJ. The renin-angiotensin system - a therapeutic target in COVID-19? *Clin Med (Lond)* 2020; **20**: e72-e75 [PMID: 32414711 DOI: 10.7861/clinmed.2020-0146]

84 **Kuba K**, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; **11**: 875-879 [PMID: 16007097 DOI: 10.1038/nm1267]

85 **Mohamed TL**, Nguyen HT, Abdul-Hafez A, Dang VX, Dang MT, Gewolb IH, Uhal BD. Prior hypoxia prevents downregulation of ACE-2 by hyperoxia in fetal human lung fibroblasts. *Exp Lung Res* 2016; **42**: 121-130 [PMID: 27093376 DOI: 10.3109/01902148.2016.1157712]

86 **Koch A**, Kähler W, Klapa S, Grams B, van Ooij PAM. The conundrum of using hyperoxia in COVID-19 treatment strategies: may intermittent therapeutic hyperoxia play a helpful role in the expression of the surface receptors ACE2 and Furin in lung tissue *via* triggering of HIF-1α? *Intensive Care Med Exp* 2020; **8**: 53 [PMID: 32953400 DOI: 10.1186/s40635-020-00323-1]

87 **Zhang X**, Zheng J, Yan Y, Ruan Z, Su Y, Wang J, Huang H, Zhang Y, Wang W, Gao J, Chi Y, Lu X, Liu Z. Angiotensin-converting enzyme 2 regulates autophagy in acute lung injury through AMPK/mTOR signaling. *Arch Biochem Biophys* 2019; **672**: 108061 [PMID: 31356776 DOI: 10.1016/j.abb.2019.07.026]

88 **Coutard B**, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res* 2020; **176**: 104742 [PMID: 32057769 DOI: 10.1016/j.antiviral.2020.104742]

89 **Li G**, He X, Zhang L, Ran Q, Wang J, Xiong A, Wu D, Chen F, Sun J, Chang C. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. *J Autoimmun* 2020; **112**: 102463 [PMID: 32303424 DOI: 10.1016/j.jaut.2020.102463]

90 **Li Z**, Li X, Shen J, Tan H, Rong T, Lin Y, Feng E, Chen Z, Jiao Y, Liu G, Zhang L, Vai Chan MT, Kei Wu WK. Bioinformatic analysis of SMN1-ACE/ACE2 interactions hinted at a potential protective effect of spinal muscular atrophy against COVID-19-induced lung injury. *Brief Bioinform* 2021; **22**: 1291-1296 [PMID: 33190150 DOI: 10.1093/bib/bbaa285]

91 **Winkler ES**, Bailey AL, Kafai NM, Nair S, McCune BT, Yu J, Fox JM, Chen RE, Earnest JT, Keeler SP, Ritter JH, Kang LI, Dort S, Robichaud A, Head R, Holtzman MJ, Diamond MS. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat Immunol* 2020; **21**: 1327-1335 [PMID: 32839612 DOI: 10.1038/s41590-020-0778-2]

92 **Han K**, Blair RV, Iwanaga N, Liu F, Russell-Lodrigue KE, Qin Z, Midkiff CC, Golden NA, Doyle-Meyers LA, Kabir ME, Chandler KE, Cutrera KL, Ren M, Monjure CJ, Lehmicke G, Fischer T, Beddingfield B, Wanek AG, Birnbaum A, Maness NJ, Roy CJ, Datta PK, Rappaport J, Kolls JK, Qin X. Lung Expression of Human Angiotensin-Converting Enzyme 2 Sensitizes the Mouse to SARS-CoV-2 Infection. *Am J Respir Cell Mol Biol* 2021; **64**: 79-88 [PMID: 32991819 DOI: 10.1165/rcmb.2020-0354OC]

93 **Tseng YH**, Yang RC, Lu TS. Two hits to the renin-angiotensin system may play a key role in severe COVID-19. *Kaohsiung J Med Sci* 2020; **36**: 389-392 [PMID: 32492292 DOI: 10.1002/kjm2.12237]

94 **Khan A**, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, Hall R, Poirier G, Ronco JJ, Tidswell M, Hardes K, Powley WM, Wright TJ, Siederer SK, Fairman DA, Lipson DA, Bayliffe AI, Lazaar AL. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care* 2017; **21**: 234 [PMID: 28877748 DOI: 10.1186/s13054-017-1823-x]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 855-867

DOI: 10.4239/wjd.v12.i6.855

ISSN 1948-9358 (online)

MINIREVIEWS

Cardiovascular autonomic neuropathy in diabetes: Pathophysiology, clinical assessment and implications

Alice Duque, Mauro Felippe Felix Mediano, Andrea De Lorenzo, Luiz Fernando Rodrigues Jr

ORCID number: Alice Duque 0000-0003-0325-9424; Mauro Felippe Felix Mediano 0000-0001-6369-3631; Andrea De Lorenzo 0000-0001-8522-6612; Luiz Fernando Rodrigues Jr 0000-0001-7007-7431.

Author contributions: Duque A wrote the manuscript; Mediano MFF and Rodrigues Jr LF wrote and revised of manuscript; De Lorenzo A contributed article conception and writing; all authors have read and approved the final manuscript.

Conflict-of-interest statement: De Lorenzo A is an employee of the National Institute of Cardiology. Mediano MFF is an employee of Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation. Rodrigues Jr LF is an employee of the National Institute of Cardiology and of the Federal University of the State of Rio de Janeiro.

Alice Duque, Mauro Felippe Felix Mediano, Andrea De Lorenzo, Luiz Fernando Rodrigues Jr, Education and Research Department, Instituto Nacional de Cardiologia, Rio de Janeiro 22240006, RJ, Brazil

Mauro Felippe Felix Mediano, Laboratory of Clinical Research on Chagas Disease, Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation, Rio de Janeiro 21040360, RJ, Brazil

Luiz Fernando Rodrigues Jr, Department of Physiological Sciences, Biomedical Institute, Federal University of the State of Rio de Janeiro, Rio de Janeiro 22240006, RJ, Brazil

Corresponding author: Andrea De Lorenzo, PhD, Professor, Education and Research Department, Instituto Nacional de Cardiologia, Rua das Laranjeiras 374, Rio de Janeiro 22240006, RJ, Brazil. andlorenzo@hotmail.com

Abstract

Cardiovascular autonomic neuropathy (CAN) is a debilitating condition that mainly occurs in long-standing type 2 diabetes patients but can manifest earlier, even before diabetes is diagnosed. CAN is a microvascular complication that results from lesions of the sympathetic and parasympathetic nerve fibers, which innervate the heart and blood vessels and promote alterations in cardiovascular autonomic control. The entire mechanism is still not elucidated, but several aspects of the pathophysiology of CAN have already been described, such as the production of advanced glycation end products, reactive oxygen species, nuclear factor kappa B, and pro-inflammatory cytokines. This microvascular complication is an important risk factor for silent myocardial ischemia, chronic kidney disease, myocardial dysfunction, major cardiovascular events, cardiac arrhythmias, and sudden death. It has also been suggested that, compared to other traditional cardiovascular risk factors, CAN progression may have a greater impact on cardiovascular disease development. However, CAN might be subclinical for several years, and a late diagnosis increases the mortality risk. The duration of the transition period from the subclinical to clinical stage remains unknown, but the progression of CAN is associated with a poor prognosis. Several tests can be used for CAN diagnosis, such as heart rate variability (HRV), cardiovascular autonomic reflex tests, and myocardial scintigraphy. Currently, it has already been described that CAN could be detected even during the subclinical stage through a reduction in HRV, which is a non-invasive test with a lower operating cost. Therefore, considering that diabetes mellitus is a global epidemic and that

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Brazil

Peer-review report's scientific quality classification

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

Received: February 28, 2021
Peer-review started: February 28, 2021
First decision: March 30, 2021
Revised: April 5, 2021
Accepted: May 20, 2021
Article in press: May 20, 2021
Published online: June 15, 2021

P-Reviewer: Shi J
S-Editor: Gao CC
L-Editor: A
P-Editor: Ma YJ

diabetic neuropathy is the most common chronic complication of diabetes, the early identification and treatment of CAN could be a key point to mitigate the morbidity and mortality associated with this long-lasting condition.

Key Words: Cardiovascular autonomic neuropathy; Cardiac autonomic neuropathy; Diabetes mellitus; Heart rate variability; Sympathetic autonomic nervous system; Parasym-pathetic autonomic nervous system

Core Tip: Cardiovascular autonomic neuropathy (CAN) is an important risk factor for cardiovascular events. However, CAN may be subclinical for several years, worsening its potential contribution to increased mortality due to late diagnosis. Even during the subclinical stage, CAN could be detected through reduction in heart rate variability, a non-invasive test. Therefore, considering that diabetes mellitus is a global epidemic and that diabetic neuropathy is the most common chronic complication of diabetes, the early identification and treatment of CAN could be a key point to mitigate the morbidity and mortality impact from this long-lasting condition.

Citation: Duque A, Mediano MFF, De Lorenzo A, Rodrigues Jr LF. Cardiovascular autonomic neuropathy in diabetes: Pathophysiology, clinical assessment and implications. *World J Diabetes* 2021; 12(6): 855-867
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/855.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.855

INTRODUCTION

Cardiovascular autonomic neuropathy (CAN) is a microvascular complication defined as the impairment of cardiovascular autonomic control in persons with diabetes, with no other causes[1]. The prevalence of CAN varies from nearly 2% in patients with newly diagnosed or well-controlled diabetes, up to 60% of patients with long-standing type 2 diabetes mellitus and 90% of pancreas transplantation candidates with type 1 diabetes[2,3]. The heterogeneity of evaluation methods used to classify CAN is a possible cause of this wide variation in prevalence, making it difficult to compare epidemiological data across different studies. CAN prevalence also increases with age, duration of diabetes, and poor glycemic control[4].

Despite CAN manifesting as a subclinical condition for several years until the development of symptoms, it is a risk factor for silent myocardial ischemia, chronic kidney disease, myocardial dysfunction, major cardiovascular events, cardiac arrhythmias, and sudden death. Moreover, it is associated with increased morbidity and mortality risk and poor long-term diabetes prognosis[5-8]. The etiology of CAN is multifactorial, and several conditions are associated with CAN, such as hyper-glycemia, insulin resistance, prediabetes, obesity, hypertension, dyslipidemia, metabolic syndrome, and obstructive sleep apnea (OSA). However, it is mainly recognized as a major complication of type 1 and type 2 diabetes mellitus[8], since diabetic neuropathies are the most prevalent chronic microvascular complications of diabetes. Of these, autonomic neuropathies (mainly CAN) and distal symmetric polyneuropathy are the most studied to date[3,9].

An increase in the incidence of CAN is expected to occur due to the progression of diabetes as a global epidemic[10,11]. In 2019, diabetes mellitus affected 463 million people worldwide. This scenario is predicted to grow to over 592 million by 2035; based on the International Diabetes Federation, this number will rise to 700 million (10.9% prevalence) by 2045[12,13]. These projections are worrying considering that, in 2016, diabetes was directly responsible for 1.6 million deaths, representing the seventh leading cause of death worldwide[14]. In addition, diabetes commonly coexists with obesity, and nearly 85% of people with diabetes are type 2 diabetics; of those, 90% are obese or overweight[15]. The burden of these chronic diseases leads to a cardiome-tabolic epidemic, with a staggering increase in the global prevalence of diabetes mellitus, obesity, and metabolic syndrome[16-18]. Therefore, early identification and

treatment of CAN could be a key point to minimize the morbidity and mortality associated with this long-lasting pandemic. The aim of this study was to review the latest content on the epidemiology, pathophysiology, and clinical assess-ment of CAN and to encourage healthcare workers to be aware of this clinical entity, considering that CAN is still an under-recognized condition[19].

CAN IN DIABETES

Definition

CAN is a debilitating condition that occurs mainly among diabetic patients, especially those with a long duration of diabetes[19], but can manifest earlier, even before the diagnosis of diabetes[20]. Among its clinical manifestations, resting tachycardia, orthostatic hypotension, light-headedness, visual impairment, syncope, and exercise intolerance are the most common[21,22]. In 1892, Eichhorst suggested that persistent tachycardia in diabetic individuals may be due to damage to the vagus nerve[23]. Bradbury and Eggleston[24] first described the clinical syndrome of orthostatic hypotension and orthostatic tachycardia in 1925, and in 1945, Rundles described these physiological abnormalities as manifestations of diabetic neuropathy[25,26]. Since 1980, several studies have evaluated cardiac autonomic denervation as a possible late-stage complication of CAN and demonstrated that it is associated with increased mortality[23,26-28].

Total cardiac denervation — the loss of sympathetic and parasympathetic innervation — is not frequent, but can occur as a result of diabetic neuropathy and, in turn, results in a blunted heart rate response. Vagal denervation is usually more common and occurs at an earlier stage before sympathetic denervation. Thus, it reverberates in abnormalities of normal heart rate variation and vascular dynamics, which are regulated by the sympathetic autonomic nervous system (SANS) and parasympathetic autonomic nervous system (PANS)[2,20,23]. The interaction and the equilibrium between SANS and PANS result in sympathovagal balance, which is responsible for modulating the sinus node; promoting adjustments of heart rate; controlling chronotropism, dromotropism, bathmotropism, and inotropism; altering the systolic and diastolic volumes; and promoting the control of vascular smooth muscle cells, contributing to peripheral vascular resistance[29-31].

Chronic modifications in the existing equilibrium between the SANS and PANS therefore cause autonomic dysfunction. The mechanisms of autonomic dysfunction are complex and multifactorial, involving degenerative, inflammatory, ischemic, and metabolic abnormalities, which compromise the intrinsic cardiac innervation as well as other structures of the autonomic nervous system[4,32,33]. As cardiovascular autonomic dysfunction is potentially arrhythmogenic, it may predispose to atrial and ventricular arrhythmias and sudden cardiac death[8,34,35]. Although CAN progression is currently considered an independent prognostic factor for cardio-vascular disease[36], it is frequently considered a subclinical condition, which may aggravate its potential contribution to the increased probability of mortality due to late diagnosis. Therefore, it is important to understand the pathophysiological mechanisms that trigger CAN, as well as which clinical assessments are currently available and recommended, in order to contribute to morbidity and mortality reduction associated to CAN[37].

Pathophysiology

CAN results from lesions of the autonomic nerve fibers that innervate the heart and blood vessels, promoting abnormalities in cardiovascular autonomic control[4]. The pathophysiological mechanism responsible for this lesion is multifactorial. Although the mechanisms associated with CAN development remain uncertain in their entirety, the main mechanism is hyperglycemia. Hyperglycemia directly favors an increase in the production of reactive oxygen species (ROS) and advanced glycation end products (AGEs), which are a heterogeneous group of compounds[7,32].

The formation of AGEs occurs mainly due to the Maillard reaction, which is a non-enzymatic reaction between the carbonyl groups of reducing sugars and free amino groups of proteins, and depends directly on the concentration of glucose[38]. This process, which takes weeks to months, is reversible in the early phases, but becomes irreversible in its final stage. After its formation, AGEs accumulate inside and outside the cells. AGEs have been described as being able to bind to receptors for AGEs (RAGE), stimulating phosphatidylinositol-3 kinase (PI3-K) and mitogen-activated protein kinases (MAPK), and, consequently, activating nuclear factor kappa B (NF-κB)

[39].

NF-κB enhances the stimulation of RAGE expression in the cell membrane of cardiomyocytes, neurons, adipocytes, vascular cells, immune cells, glomerular epithelial cells, and lung epithelial cells, promoting a positive feedback response. Moreover, this transcription factor amplifies the production of tumor necrosis factor αand interleukin 6, which are pro-inflammatory cytokines, and vascular cell adhesion molecule 1, which promotes transendothelial migration of leukocytes[39-41]. In addition to hyperglycemia caused by type 1 and type 2 diabetes, other factors can also increase the production of NF-κB and pro-inflammatory cytokines, such as fatty acid accumulation, obesity, and atherosclerosis[40,42]. NF-κB also plays a crucial role in obesity-induced inflammation and insulin resistance[42].

AGE/RAGE signaling promotes nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation, expanding the production of ROS and oxidative stress [39]. Oxidative stress is defined as an imbalance between ROS production and antioxidant defense systems (superoxide dismutase, catalase, and glutathione peroxidase). Thus, the complex of NADPH oxidase produces superoxide, which, together with hydroxyl radicals, singlet oxygen, and hydrogen peroxide, represent ROS. They have the ability to act as highly reactive free radicals, promote protein, lipid, and nucleic acid oxidation, and induce cellular damage. Moreover, the increase in oxidative stress activates NF-κB and, consequently, increases the expression of RAGEs in the cell membrane, emphasizing AGE/RAGE signaling and promoting positive feedback[39,43].

In particular, it has been demonstrated that plasma superoxide anion is a primary biomarker of oxidative stress, and its increased production works as a predictor of cardiac autonomic dysfunction progression and even all-cause mortality[44]. Thus, chronic increased oxidative stress has a dangerous impact on the autonomic fibers and β-pancreatic cells, triggering the insulin resistance process and the development of type 2 diabetes mellitus. In addition, the increase in oxidative stress is associated not only with the progression of diabetes, but also with dyslipidemia, atherosclerosis, cancer, and cardiovascular diseases[43-45].

Another component that may be associated with the pathogenesis of CAN is OSA [46]. OSA can be defined as a syndrome marked by frequent pauses in breathing during sleep that is usually accompanied by loud snoring, which occurs due to upper airway collapse[47,48]. Although the exact mechanism remains obscure, this disorder can lead to intermittent hypoxia that increases oxidative stress, contributing to CAN development[46]. Moreover, OSA is associated with increased cardiovascular morbidity and is commonly present in diabetic patients[46,48].

CAN, diabetes, and mortality

Several studies have demonstrated the relationship between CAN and increased morbidity and mortality in patients with diabetes[49-51]. In 1991, Ewing *et al*[50] investigated the association between QT interval and corrected QT interval (QTc) length and sudden death in patients with diabetes. They showed that among 71 diabetic subjects, 13 died unexpectedly within three years of follow-up, and the QT and QTc intervals were significantly increased in these 13 participants. Thus, QT and QTc interval prolongation were associated with an increased risk of unexpected death in diabetic individuals with CAN[50].

Thereafter, in 2005, the Rochester diabetic neuropathy study (RDNS) evaluated CAN and the risk factors for sudden cardiac death. Suarez *et al*[52] demonstrated an association between an increase in the QTc interval and sudden cardiac death *via* univariate analysis, but this significance was not observed in the multivariate analysis. Thus, they suggested that other conditions could have influenced this worse prognosis, such as nephropathy. This microvascular complication could be a marker of generalized vascular dysfunction and was marked as an independent risk factor for sudden death in the RDNS study.

Despite the RDSN findings, the Diabetes Heart Study demonstrated that QTc interval predicted all-cause and cardiovascular disease mortality in participants with type 2 diabetes mellitus[53], confirming the results previously obtained by Ewing *et al* [50]. In addition, in 2010, Pop-Busui *et al*[54] evaluated the mortality risk in participants with CAN and reported that CAN participants had a twofold all-cause mortality risk compared to individuals without CAN.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial demonstrated that participants with CAN had similar mortality rates when following both standard and intensive treatments for glycemic control, suggesting that severe glycemic control could promote hypoglycemia and increase the probability of mortality in diabetic patients[54]. Another study developed by Tang *et al*[51] based on

the ACCORD trial evaluated the effects of intensive treatment of hyperglycemia, hypertension, and dyslipidemia as a prevention strategy to reduce cardiovascular events. Considering that these three conditions are important cardiovascular risk factors that must be controlled, the study showed that intensive control of blood pressure and glycemia promotes protective effects on CAN[51]. These findings reinforce that poor glycemic control, the duration of diabetes, and lifestyle factors play a crucial role in CAN development[19]. A brief description of relevant studies on CAN and diabetes is provided in Table 1.

Clinical assessment

Currently, it is estimated that about 50% of people with diabetes mellitus remain undiagnosed[46] and, among those diagnosed, the diagnosis usually happens very late, approximately 20 years after the onset of the disease[55]. Thus, considering that CAN may present even before the onset of diabetes, it is a markedly underdiagnosed and underestimated microvascular complication[20,56]. The natural progression of CAN comprises an asymptomatic, subclinical, and reversible phase, which represents the initial stage. Subsequently, CAN progresses to more advanced stages, with symptoms and a greater impairment of cardiac autonomic fibers[20,33,46].

Autonomic neuropathy battery tests, known as cardiovascular autonomic reflex tests (CARTs), are used to assess stages of and monitor the progression of CAN. They are composed of tests that evaluate autonomic responses through changes in heart rate, blood pressure, and sudomotor responses after several maneuvers[22]. Some of the available tests were described by Ewing *et al*[57-60] in the 1970s and the 1980s and are known as Ewing's Battery composed of five tests as follows: Valsalva maneuver, heart rate response to standing (30:15 ratio), heart rate response to deep breathing (maximum-minimum heart rate), blood pressure response to standing up (orthostatic hypotension test), and blood pressure response to sustained handgrip (isometric handgrip test)[60].

EWING'S BATTERY

Heart rate response to deep breathing

The deep breathing test is associated with respiratory arrhythmia and evaluates PANS function. Patients are asked to breathe at a rate of six times per minute, with approximately 5 s of inhalation and 5 s of exhalation per breath. The examiner must calculate the difference between the average of the largest accelerations (inspiration time) and the average of the largest decelerations (expiration time), and the expected result is at least 10 breaths/min to 15 breaths/min, which can decrease with aging. Moreover, it allows the calculation of the expiratory-inspiratory ratio (E:I ratio), which represents the ratio of the longest RR interval during expiration divided by the shortest RR interval during inspiration from five cycles. The result should be at least 1.2 in young individuals[4,61,62].

Heart rate response to standing

The heart rate response to standing is referred to as the 30:15 ratio, another test designed to assess PANS function. The protocol consists of asking the patient to rest in the supine position for a specified amount of time, then to change this posture to an erect position. It is calculated based on the ratio between the longest RR interval (between the 20th and 40th beat, around the 30th heartbeat) and the shortest RR interval (between the 5th and 25th beat, around the 15th heartbeat) after standing up. The RR intervals are measured using an electrocardiogram record, and the result should be at least 1.04. In addition, sinus tachycardia, neurocardiogenic syncope, and abnormalities in baroreceptor function could also be detected with this test[4,61,62].

Valsalva maneuver

The Valsalva maneuver represents voluntary forced expiration against resistance. The test is performed with an electrocardiogram record and evaluates the PANS function with high sensitivity. During expiration, the patient should maintain a mercury column at 40 mmHg for 15 s. Subsequently, physiological tachycardia commonly occurs. The electrocardiogram remains recording for 30 s to 45 s, when physiological bradycardia commonly occurs. The ratio of the shortest RR intervals (maximum heart rate) divided by the longest RR intervals (slowest heart rate) represents the Valsalva ratio, and values below 1.21 are considered abnormal results[4,62].

Table 1 Characteristics of different studies evaluating cardiovascular autonomic neuropathy and diabetes

Study	Ref.	Sample size and type of study	CAN assessment	Main findings
Pittsburgh Epidemiology of Diabetes Complications Study III	Maser *et al* [49], 1990	168 participants with type 1 diabetes; Cross-sectional study	Heart rate response to deep breathing, 30:15 ratio and Valsalva maneuver	The association ofCAN with increased cardiovascular risk factors may explain the high mortality of CAN patients
EURODIAB IDDM Complications Study	Kempler *et al*[85], 2002	3,007 participants with type 1 diabetes; Cross-sectional study	Orthostatic hypotension test and 30:15 ratio	CAN is associated to cardiovascular disease and vascular factors may have an important role in the pathogenesis of CAN
EURODIAB Prospective Complications Study	Witte *et al* [86], 2005	956 participants with type 1 diabetes; Prospective cohort study (mean follow-up of 7 yr)	Orthostatic hypotension test and 30:15 ratio	Glycated hemoglobin level, hypertension, distal symmetrical polyneuropathy and retinopathy, predict the risk of CAN development
MONICA/KORA Augsburg Cohort Study	Ziegler *et al*[87], 2008	1720 participants (1560 non-diabetic and 160 diabetic subjects); Prospective cohort study (mean follow-up of 9 yr)	HRV, corrected QT interval and QT dispersion (difference between the longest and shortest QT intervals in 12-lead electrocardiogram)	Prolonged corrected QT interval is an independent predictor of mortality in the non-diabetic anddiabetic population, while reduced HRV appears to be a prognostic index only in the presence of diabetes
ACCORD Trial	Pop-Busui *et al*[54], 2010	10251 participants with type 2 diabetes; Clinical Trial	HRV, resting heart rate and QT index (observed/predicted QT duration)	CAN patients had a 1.55-2.14 increased relative risk of all-cause mortality compared to those without CAN
First Joslin Kidney Study	Orlov *et al* [88], 2015	370 participants with type 1 diabetes; Prospective cohort study (mean follow-up of 14 yr)	Heart rate response to deep breathing	CAN is a strong independent predictor of the long-term risk of early decline of renal function
ACCORD Trial	Tang *et al* [51], 2021	7725 participants with type 2 diabetes; Clinical Trial	HRV and QT index	The intensive blood pressure and glycemic control demonstrated favorable impact in patients with CAN

CAN: Cardiovascular autonomic neuropathy; HRV: Heart rate variability.

Blood pressure response to standing up

The blood pressure response to standing up is the so-called orthostatic hypotension test or postural hypotension test. This test evaluates variations in blood pressure between the rest period and after standing for three min, corresponding to the evaluation of the SANS function. A decrease in systolic blood pressure ≥ 20 mmHg and/or diastolic blood pressure ≥ 10 mmHg upon standing should be considered as an abnormal result. Moreover, several other symptoms or conditions can be identified with this test, such as weakness, faintness, dizziness, and visual impairment[4,62,63].

Blood pressure response to sustained handgrip

The isometric handgrip test consists of pressing the handgrip with nearly 30% of the maximum contraction strength for 3-5 min. This maneuver could be performed with the dominant arm and/or the non-dominant arm and is supposed to promote an increase in diastolic blood pressure. Blood pressure is measured in the contralateral arm, and an increase of at least 15 mmHg between the rest and peak effort values is expected. This test mainly evaluates the SANS response due to isometric exercise[4,62].

The Ewing's Battery tests represent a framework of CAN and its severity assessment in a simple, fast, and non-invasive manner[60]. Based on the diagnostic tests and clinical stages, CAN could be classified as follows: subclinical stage, possible or early CAN [decreased heart rate variability (HRV) or one abnormal cardiovagal test from CARTs], definite or confirmed CAN (presence of two or more abnormal CARTs results and often accompanied by resting tachycardia), and severe or advanced CAN (presence of definite or confirmed CAN and orthostatic hypotension, often accompanied by evidence of cardiomyopathy with left ventricular dysfunction on echocardiography and silent myocardial ischemia)[8,10]. Symptomatic CAN may be considered as severe or advanced CAN with exercise intolerance, postural dizziness, palpitations, or presyncope[8].

According to these stages, Ewing *et al*[60] suggested that early involvement, definite involvement, and severe involvement should be interpreted as early parasympathetic, definite parasympathetic, and parasympathetic with additional sympathetic compromise, respectively. In addition, each Ewing's test can be scored as 0 for a

normal result, 0.5 for a borderline result, and 1 for an abnormal result. Therefore, a total score of 0-5 can be attributed to the standard battery performance, as previously described[60,64]. The progression of CAN stages is associated with a worse prognosis, emphasizing the need for tests for early diagnosis[8].

However, there are some criticisms about the feasibility of Ewing's tests, such as the difficulty in performing some tests in patients with osteomioarticular conditions or other mobility difficulties. Moreover, the results of the orthostatic hypotension test could not be reliable in patients with fluid retention, and the Valsalva maneuver directly depends on the patient's comprehension[65]. On the other hand, a potentially useful framework that overcomes these limitations is nuclear imaging, despite the high cost being a major limitation. It is a functional assessment tool used to evaluate presynaptic sympathetic nervous system function using myocardial scintigraphy[63].

Myocardial scintigraphy

Myocardial scintigraphy with 123I-metaiodobenzylguanidine (^{123}I-MIBG) allows for the evaluation of sympathetic presynaptic integrity[63]. After being injected, ^{123}I-MIBG diffuses into synaptic spaces and is absorbed into pre-synaptic terminals just like norepinephrine. Thus, considering that ^{123}I-MIBG is a false neurotransmitter, it is not catabolized and allows the visualization and quantification of norepinephrine transporter-1 function and, consequently, cardiac sympathetic innervation[66]. Several studies demonstrated abnormalities in sympathetic innervation in diabetic patients through myocardial scintigraphy with ^{123}I-MIBG[67-70]. In addition to CAN evaluation in diabetic patients, cardiac sympathetic imaging has other potential clinical applications, such as heart failure, transplantation, ischemic heart disease, and chemotherapy-induced cardiotoxicity[66].

The clinical use of ^{123}I-MIBG for cardiac and non-cardiac imaging has already been approved in some countries. However, this technique has an elevated cost and its clinical use is still limited[71]; therefore, CARTs continue to be the most commonly used methods for CAN diagnosis[36], providing a quick and non-invasive assessment of cardiac autonomic function at a lower operating cost[72], despite the increased sensitivity of ^{123}I-MIBG scintigraphy[69].

HRV

The HRV test is a cost-effective measurement based on the RR interval oscillation analysis of consecutive heartbeats. The duration of the RR intervals is not fixed, and reflects the combined performance of the SANS and PANS[73]. Thus, HRV is a marker of cardiac autonomic function, which is suitable for cardiovascular risk stratification. Its reduction is associated with increased cardiovascular risk[63,73]. In addition, HRV is recognized as a predictive factor of silent myocardial infarction and postmyocardial infarction mortality[36].

HRV can be evaluated using linear or nonlinear methods. The nonlinear methods comprise the detrended fluctuation analysis, Hurst exponent, fractal dimension, and Lyapunov exponent. Although these indices are good morbidity and mortality markers, they require long periods of analysis. On the other hand, linear methods can be evaluated in a short period and are divided into two groups: those analyzed in the time domain and those analyzed in the frequency domain[74]. The parameters of these domains are listed in Table 2.

Despite the fact that HRV indices and their respective interpretations are well-established in the literature, there is still no standardization of their reference values. In 1996, the European Society of Cardiology and the North American Society of Pacing and Electrophysiology published guidelines with standardized values of HRV measurements and their clinical associations. However, some of the ranges came from studies with small sample sizes, and the values were not adjusted for potential confounders, such as sex, age, or environmental factors. Thus, they should be considered as estimate values that requires more robust physiological and clinical validation[75].

Another criticism of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology guidelines is the statement that HRV is a simple tool. Although the technique has spread mainly due to devices that provide an automated measurement of HRV, the guidelines generate complex parameters and should be interpreted with caution in order to avoid incorrect data conclusions and extrapolations[33]. Nevertheless, despite criticism and the absence of standardized reference values, HRV remains a method widely associated with the body's self-regulatory capacity and the early identification of autonomic alterations and increased cardiovascular risk[76-78]. Moreover, HRV has been reported as a tool to identify cardiovascular risk, even in individuals without previous cardiovascular diseases[77,

Table 2 Heart rate variability time and frequency domain measures[89,90]

	Linear indices - time domain	
Parameters	**Abbreviation meaning**	**Interpretation**
MNN (ms)	Mean of NN intervals	Long RR intervals are related to a lower heart rate, while short RR intervals denote a high heart rate. It reflects SANS and PANS modulations
SDNN (ms)	Standard deviation of all NN intervals	Reflects the activity of both SANS and PANS
rMSSD (ms)	The square root of the mean squared differences of successive NN intervals	Reflects the PANS activity
NN50 (count)	Number of interval differences of successive NN intervals greater than 50 ms	Reflects the PANS activity
pNN50 (%)	Percentage of successive RR intervals that differ by more than 50 ms	The proportion of NN50 divided by total number of NN, which also represents the PANS activity
	Linear indices - frequency domain	
ULF (ms^2, Hz, %)	Ultra low frequency	Frequency range: 0-0.003 Hz. Commonly, it is not present in HRV results
VLF (ms^2, Hz, %)	Very low frequency	Frequency range: 0.003-0.04 Hz. It is related to renin-angiotensin-aldosterone system, thermoregulation, peripheral vasomotor tonus and PANS activity
LF (ms^2, Hz, nu, %)	Low frequency	Frequency range: 0.04-0.15 Hz. It represents the SANS and PANS activity, with a predominance of SANS influence
HF (ms^2, Hz, nu, %)	High frequency	Frequency range: 0.15-0.4 Hz. It represents the PANS activity
LF/HF	Ratio of LF-to-HF power	So-called sympathovagal index. It represents the sympathovagal balance, the autonomic state resulting from the SANS and PANS influences
Total power (ms^2)	Total power	It reflects both SANS and PANS influences, representing the components with frequency range ≤ 0.4 Hz

SANS: Sympathetic autonomic nervous system; PANS: Parasympathetic autonomic nervous system; LF: Low frequency; HF: High frequency; ULF: Ultra low frequency; VLF: Very low frequency; MNN: Mean of NN; SDNN: Standard deviation of all NN.

79].

CRITICAL REFLECTION

Diabetes mellitus is a global epidemic[46], and diabetic neuropathy is the most common chronic complication[63]. Among the types of diabetic neuropathy, CAN is one of the most studied and disabling conditions[19]. Considering that CAN is a major marker for silent myocardial ischemia, myocardial dysfunction, cardiac arrhythmias, and sudden death[5-8], it is surprising that CAN is still an under-investigated condition in patients with diabetes[1].

CAN may present in a subclinical form for many years while the parasympathetic denervation process already occurs in diabetic patients[80]. Moreover, CAN is associated with increased morbidity and mortality risks[4]. However, there is no universal standard method for detecting CAN, and it is suggested that more than one test should be conducted to enhance the sensitivity and reliability of CAN diagnosis [19]. In this setting, several tests with different degrees of accuracy, such as CARTs, HRV, and nuclear imaging, are available[61,69,81,82].

According to the position statement of the American Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE), CAN screening should be performed at the time of diagnosis in patients with type 2 diabetes mellitus and five years after diagnosis in patients with type 1 diabetes mellitus. Nevertheless, there are still some controversies regarding the guidelines for CAN screening. For instance, the position statement of the American Diabetes Association (ADA) considers a patient eligible for CAN assessment only if they have microvascular complications and/or hypoglycemia unawareness. On the other hand, the Italian Society of Diabetology (SID) and the Italian Association of Clinical Diabetologists (AMD) reported that patients should be evaluated if they have high cardiovascular risk and complications, while the Toronto Consensus emphasizes that screening for symptoms and signs of CAN should be universal[10].

There is also disagreement about the use of HRV tests for the diagnosis of CAN. According to the ADA, SID, and AMD statements, this technique is mainly used for research purposes. In contrast, the AACE, ACE, and Toronto Consensus recognize the clinical and prognostic value of the HRV test[10]. Despite the importance of early detection, there is no harmonized definition of CAN, and CAN is frequently diagnosed late[83].

Therefore, early recognition of CAN is essential to minimize the risk of morbidity and mortality in patients with diabetes. CARTs, HRV, and the ^{123}I-mIBG myocardial scintigraphy should be used in combination for the CAN diagnosis in diabetic patients [63,84]. A harmonized definition among scientific societies is urgently needed to recommend standardized methods for CAN screening in patients with low, medium, and high cardiovascular risk. In view of the autonomic alterations associated with hyperglycemia, the early identification of sympathovagal imbalance in CAN may change treatment strategies for diabetic patients. Moreover, HRV analysis may be used as a potential tool to identify the first signs of CAN, even in asymptomatic individuals [84].

CONCLUSION

Although CAN is considered a condition associated with increased risks of morbidity and mortality, there are still many disagreements regarding the recommendations in the CAN guidelines. The existence of complex mechanisms, the wide variety of tools for assessing CAN, and the lack of a harmonized definition among the scientific societies contribute to the reduced clinical investigation of this complication, which can increase the risk of silent myocardial ischemia, myocardial dysfunction, cardiac arrhythmias, and sudden death.

CAN assessment methodologies (HRV, CARTs, and ^{123}I-mIBG myocardial scintigraphy) need to become more available, widely accessible, and easy to interpret. Considering that CAN is an under-recognized condition, it is also necessary to stimulate the discussion about this microvascular complication in college or university programs in the healthcare field. Investing in education and stimulating the assessment of this complication can be a promising key point for early identification and reducing morbimortality of CAN, mainly in the current scenario of diabetes and cardiometabolic epidemics.

REFERENCES

1 **Spallone V**, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempler P, Hilsted J, Tesfaye S, Low P, Valensi P; Toronto Consensus Panel on Diabetic Neuropathy. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev* 2011; **27**: 639-653 [PMID: 21695768 DOI: 10.1002/dmrr.1239]

2 **Vinik AI**, Erbas T, Casellini CM. Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease. *J Diabetes Investig* 2013; **4**: 4-18 [PMID: 23550085 DOI: 10.1111/jdi.12042]

3 **Pop-Busui R**. What do we know and we do not know about cardiovascular autonomic neuropathy in diabetes. *J Cardiovasc Transl Res* 2012; **5**: 463-478 [PMID: 22644723 DOI: 10.1007/s12265-012-9367-6]

4 **Vinik AI**, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation* 2007; **115**: 387-397 [PMID: 17242296 DOI: 10.1161/CIRCULATIONAHA.106.634949]

5 **Razanskaite-Virbickiene D**, Danyte E, Mockeviciene G, Dobrovolskiene R, Verkauskiene R, Zalinkevicius R. Can coefficient of variation of time-domain analysis be valuable for detecting cardiovascular autonomic neuropathy in young patients with type 1 diabetes: a case control study. *BMC Cardiovasc Disord* 2017; **17**: 34 [PMID: 28103812 DOI: 10.1186/s12872-016-0467-0]

6 **Astrup AS**, Tarnow L, Rossing P, Hansen BV, Hilsted J, Parving HH. Cardiac autonomic neuropathy predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. *Diabetes Care* 2006; **29**: 334-339 [PMID: 16443883 DOI: 10.2337/diacare.29.02.06.dc05-1242]

7 **Fisher VL**, Tahrani AA. Cardiac autonomic neuropathy in patients with diabetes mellitus: current perspectives. *Diabetes Metab Syndr Obes* 2017; **10**: 419-434 [PMID: 29062239 DOI: 10.2147/DMSO.S129797]

8 **Williams SM**, Eleftheriadou A, Alam U, Cuthbertson DJ, Wilding JPH. Cardiac Autonomic Neuropathy in Obesity, the Metabolic Syndrome and Prediabetes: A Narrative Review. *Diabetes Ther* 2019; **10**: 1995-2021 [PMID: 31552598 DOI: 10.1007/s13300-019-00693-0]

9 **Pop-Busui R**, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care*

2017; **40**: 136-154 [PMID: 27999003 DOI: 10.2337/dc16-2042]
10 **Spallone V**. Update on the Impact, Diagnosis and Management of Cardiovascular Autonomic Neuropathy in Diabetes: What Is Defined, What Is New, and What Is Unmet. *Diabetes Metab J* 2019; **43**: 3-30 [PMID: 30793549 DOI: 10.4093/dmj.2018.0259]
11 **Eleftheriadou A**, Williams S, Nevitt S, Brown E, Roylance R, Wilding JPH, Cuthbertson DJ, Alam U. The prevalence of cardiac autonomic neuropathy in prediabetes: a systematic review. *Diabetologia* 2021; **64**: 288-303 [PMID: 33164108 DOI: 10.1007/s00125-020-05316-z]
12 **Hu FB**, Satija A, Manson JE. Curbing the Diabetes Pandemic: The Need for Global Policy Solutions. *JAMA* 2015; **313**: 2319-2320 [PMID: 25996138 DOI: 10.1001/jama.2015.5287]
13 **Williams R**, Colagiuri S, Chan J, Gregg E, Ke C, Lim L-L. IDF Atlas 9th Edition 2019. Brussels: Belgium, 2019
14 **World Health Organization**. Diabetes. Key facts. [cited 18 February 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes
15 **Haththotuwa RN**, Wijeyaratne CN, Senarath U. Chapter 1 - Worldwide epidemic of obesity. In: Mahmood TA, Arulkumaran S, Chervenak FA. Obesity and Obstetrics (Second Edition). Elsevier, 2020: 3-8
16 **Eckel RH**, Blaha MJ. Cardiometabolic Medicine: A Call for a New Subspeciality Training Track in Internal Medicine. *Am J Med* 2019; **132**: 788-790 [PMID: 30871919 DOI: 10.1016/j.amjmed.2019.02.027]
17 **Lopez-Jaramillo P**, Lahera V, Lopez-Lopez J. Epidemic of cardiometabolic diseases: a Latin American point of view. *Ther Adv Cardiovasc Dis* 2011; **5**: 119-131 [PMID: 21406494 DOI: 10.1177/1753944711403189]
18 **Reiter-Brennan C**, Cainzos-Achirica M, Soroosh G, Saxon DR, Blaha MJ, Eckel RH. Cardiometabolic medicine - the US perspective on a new subspecialty. *Cardiovasc Endocrinol Metab* 2020; **9**: 70-80 [PMID: 32803138 DOI: 10.1097/XCE.0000000000000224]
19 **Pan Q**, Li Q, Deng W, Zhao D, Qi L, Huang W, Ma L, Li H, Li Y, Lyu X, Wang A, Yao H, Guo L, Xing X. Prevalence and Diagnosis of Diabetic Cardiovascular Autonomic Neuropathy in Beijing, China: A Retrospective Multicenter Clinical Study. *Front Neurosci* 2019; **13**: 1144 [PMID: 31708736 DOI: 10.3389/fnins.2019.01144]
20 **Rolim LC**, Sá JR, Chacra AR, Dib SA. Diabetic cardiovascular autonomic neuropathy: risk factors, clinical impact and early diagnosis. *Arq Bras Cardiol* 2008; **90**: e24-e31 [PMID: 18516377 DOI: 10.1590/s0066-782x2008000400014]
21 **Serhiyenko VA**, Serhiyenko AA. Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment. *World J Diabetes* 2018; **9**: 1-24 [PMID: 29359025 DOI: 10.4239/wjd.v9.i1.1]
22 **Agashe S**, Petak S. Cardiac Autonomic Neuropathy in Diabetes Mellitus. *Methodist Debakey Cardiovasc J* 2018; **14**: 251-256 [PMID: 30788010]
23 **Watkins PJ**, Mackay JD. Cardiac denervation in diabetic neuropathy. *Ann Intern Med* 1980; **92**: 304-307 [PMID: 7356218 DOI: 10.7326/0003-4819-92-2-304]
24 **Bradbury S**, Eggleston C. Postural hypotension: A report of three cases. *Am Heart J* 1925; **1**: 73-86 [DOI: 10.1016/s0002-8703(25)90007-5]
25 **Rundles RW**. Diabetic Neuropathy: General Review with Report of 125 Cases. *Medicine* 1945; **24**: 111-160
26 **Mackay JD**, Page MM, Cambridge J, Watkins PJ. Diabetic autonomic neuropathy. The diagnostic value of heart rate monitoring. *Diabetologia* 1980; **18**: 471-478 [PMID: 7418957 DOI: 10.1007/BF00261703]
27 **Lloyd-Mostyn RH**, Watkins PJ. Total cardiac denervation in diabetic autonomic neuropathy. *Diabetes* 1976; **25**: 748-751 [PMID: 955302 DOI: 10.2337/diab.25.9.748]
28 **Watkins PJ**, Edmonds ME. Sympathetic nerve failure in diabetes. *Diabetologia* 1983; **25**: 73-77 [PMID: 6628876 DOI: 10.1007/BF00250890]
29 **Boutagy NE**, Sinusas AJ. Recent Advances and Clinical Applications of PET Cardiac Autonomic Nervous System Imaging. *Curr Cardiol Rep* 2017; **19**: 33 [PMID: 28321682 DOI: 10.1007/s11886-017-0843-0]
30 **Sheng Y**, Zhu L. The crosstalk between autonomic nervous system and blood vessels. *Int J Physiol Pathophysiol Pharmacol* 2018; **10**: 17-28 [PMID: 29593847]
31 **Dyavanapalli J**. Novel approaches to restore parasympathetic activity to the heart in cardiorespiratory diseases. *Am J Physiol Heart Circ Physiol* 2020; **319**: H1153-H1161 [PMID: 33035444 DOI: 10.1152/ajpheart.00398.2020]
32 **Breder ISS**, Sposito AC. Cardiovascular autonomic neuropathy in type 2 diabetic patients. *Rev Assoc Med Bras (1992)* 2019; **65**: 56-60 [PMID: 30758421 DOI: 10.1590/1806-9282.65.1.56]
33 **Colombo J**, Arora R, Depace N, Vinik A. Clinical autonomic dysfunction: Measurement, indications, therapies, and outcomes. 1st ed. Springer International Publishing, 2015: 1-452
34 **Shen MJ**, Zipes DP. Role of the autonomic nervous system in modulating cardiac arrhythmias. *Circ Res* 2014; **114**: 1004-1021 [PMID: 24625726 DOI: 10.1161/CIRCRESAHA.113.302549]
35 **Wang YC**. Rehabilitation of Patients With Neuropathies. In: Cifu DX, Lew HL. Braddom's Rehabilitation Care: A Clinical Handbook. Elsevier, 2018: 287-298.e9
36 **Yun JS**, Park YM, Cha SA, Ahn YB, Ko SH. Progression of cardiovascular autonomic neuropathy and cardiovascular disease in type 2 diabetes. *Cardiovasc Diabetol* 2018; **17**: 109 [PMID: 30071872 DOI: 10.1186/s12933-018-0752-6]
37 **Dayem SM**, Battah AA, Bohy Ael M. Cardiovascular Autonomic Neuropathy and Early

Atherosclerosis in Adolescent Type 1 Diabetic Patient. *Open Access Maced J Med Sci* 2015; **3**: 681-688 [PMID: 27275308 DOI: 10.3889/oamjms.2015.131]

38 **Singh VP**, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol* 2014; **18**: 1-14 [PMID: 24634591 DOI: 10.4196/kjpp.2014.18.1.1]

39 **Luevano-Contreras C**, Chapman-Novakofski K. Dietary advanced glycation end products and aging. *Nutrients* 2010; **2**: 1247-1265 [PMID: 22254007 DOI: 10.3390/nu2121247]

40 **Egaña-Gorroño L**, López-Díez R, Yepuri G, Ramirez LS, Reverdatto S, Gugger PF, Shekhtman A, Ramasamy R, Schmidt AM. Receptor for Advanced Glycation End Products (RAGE) and Mechanisms and Therapeutic Opportunities in Diabetes and Cardiovascular Disease: Insights From Human Subjects and Animal Models. *Front Cardiovasc Med* 2020; **7**: 37 [PMID: 32211423 DOI: 10.3389/fcvm.2020.00037]

41 **Kong DH**, Kim YK, Kim MR, Jang JH, Lee S. Emerging Roles of Vascular Cell Adhesion Molecule-1 (VCAM-1) in Immunological Disorders and Cancer. *Int J Mol Sci* 2018; **19**: 1057 [PMID: 29614819 DOI: 10.3390/ijms19041057]

42 **Kim JK**. Endothelial nuclear factor κB in obesity and aging: is endothelial nuclear factor κB a master regulator of inflammation and insulin resistance? *Circulation* 2012; **125**: 1081-1083 [PMID: 22302839 DOI: 10.1161/CIRCULATIONAHA.111.090134]

43 **Pizzino G**, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev* 2017; **2017**: 8416763 [PMID: 28819546 DOI: 10.1155/2017/8416763]

44 **Ziegler D**, Buchholz S, Sohr C, Nourooz-Zadeh J, Roden M. Oxidative stress predicts progression of peripheral and cardiac autonomic nerve dysfunction over 6 years in diabetic patients. *Acta Diabetol* 2015; **52**: 65-72 [PMID: 24898524 DOI: 10.1007/s00592-014-0601-3]

45 **Tangvarasittichai S**. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes* 2015; **6**: 456-480 [PMID: 25897356 DOI: 10.4239/wjd.v6.i3.456]

46 **Dimitropoulos G**, Tahrani AA, Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. *World J Diabetes* 2014; **5**: 17-39 [PMID: 24567799 DOI: 10.4239/wjd.v5.i1.17]

47 **World Health Organization**. Noncommunicable diseases: Obstructive sleep apnoea syndrome. Management-Screening, Diagnosis and Treatment. [cited 18 February 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/q-a-detail/noncommunicable-diseases-obstructive-sleep-apnoea-syndrome

48 **Spicuzza L**, Caruso D, Di Maria G. Obstructive sleep apnoea syndrome and its management. *Ther Adv Chronic Dis* 2015; **6**: 273-285 [PMID: 26336596 DOI: 10.1177/2040622315590318]

49 **Maser RE**, Pfeifer MA, Dorman JS, Kuller LH, Becker DJ, Orchard TJ. Diabetic autonomic neuropathy and cardiovascular risk. Pittsburgh Epidemiology of Diabetes Complications Study III. *Arch Intern Med* 1990; **150**: 1218-1222 [PMID: 2353855 DOI: 10.1001/archinte.1990.00390180056009]

50 **Ewing DJ**, Boland O, Neilson JM, Cho CG, Clarke BF. Autonomic neuropathy, QT interval lengthening, and unexpected deaths in male diabetic patients. *Diabetologia* 1991; **34**: 182-185 [PMID: 1884890 DOI: 10.1007/BF00418273]

51 **Tang Y**, Shah H, Bueno Junior CR, Sun X, Mitri J, Sambataro M, Sambado L, Gerstein HC, Fonseca V, Doria A, Pop-Busui R. Intensive Risk Factor Management and Cardiovascular Autonomic Neuropathy in Type 2 Diabetes: The ACCORD Trial. *Diabetes Care* 2021; **44**: 164-173 [PMID: 33144354 DOI: 10.2337/dc20-1842]

52 **Suarez GA**, Clark VM, Norell JE, Kottke TE, Callahan MJ, O'Brien PC, Low PA, Dyck PJ. Sudden cardiac death in diabetes mellitus: risk factors in the Rochester diabetic neuropathy study. *J Neurol Neurosurg Psychiatry* 2005; **76**: 240-245 [PMID: 15654040 DOI: 10.1136/jnnp.2004.039339]

53 **Cox AJ**, Azeem A, Yeboah J, Soliman EZ, Aggarwal SR, Bertoni AG, Carr JJ, Freedman BI, Herrington DM, Bowden DW. Heart rate-corrected QT interval is an independent predictor of all-cause and cardiovascular mortality in individuals with type 2 diabetes: the Diabetes Heart Study. *Diabetes Care* 2014; **37**: 1454-1461 [PMID: 24574343 DOI: 10.2337/dc13-1257]

54 **Pop-Busui R**, Evans GW, Gerstein HC, Fonseca V, Fleg JL, Hoogwerf BJ, Genuth S, Grimm RH, Corson MA, Prineas R; Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of cardiac autonomic dysfunction on mortality risk in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Diabetes Care* 2010; **33**: 1578-1584 [PMID: 20215456 DOI: 10.2337/dc10-0125]

55 **Sagesaka H**, Sato Y, Someya Y, Tamura Y, Shimodaira M, Miyakoshi T, Hirabayashi K, Koike H, Yamashita K, Watada H, Aizawa T. Type 2 Diabetes: When Does It Start? *J Endocr Soc* 2018; **2**: 476-484 [PMID: 29732459 DOI: 10.1210/js.2018-00071]

56 **Vinik AI**, Casellini C, Parson HK, Colberg SR, Nevoret ML. Cardiac Autonomic Neuropathy in Diabetes: A Predictor of Cardiometabolic Events. *Front Neurosci* 2018; **12**: 591 [PMID: 30210276 DOI: 10.3389/fnins.2018.00591]

57 **Ewing DJ**, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J (Clin Res Ed)* 1982; **285**: 916-918 [PMID: 6811067 DOI: 10.1136/bmj.285.6346.916]

58 **Ewing DJ**, Campbell IW, Burt AA, Clarke BF. Vascular reflexes in diabetic autonomic neuropathy. *Lancet* 1973; **2**: 1354-1356 [PMID: 4128054 DOI: 10.1016/s0140-6736(73)93323-0]

59 **Ewing DJ**, Campbell IW, Murray A, Neilson JM, Clarke BF. Immediate heart-rate response to standing: simple test for autonomic neuropathy in diabetes. *Br Med J* 1978; **1**: 145-147 [PMID: 620228 DOI: 10.1136/bmj.1.6106.145]

60 **Ewing DJ**, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 1985; **8**: 491-498 [PMID: 4053936 DOI: 10.2337/diacare.8.5.491]

61 **Bissinger A**. Cardiac Autonomic Neuropathy: Why Should Cardiologists Care about That? *J Diabetes Res* 2017; **2017**: 5374176 [PMID: 29214181 DOI: 10.1155/2017/5374176]

62 **Zygmunt A**, Stanczyk J. Methods of evaluation of autonomic nervous system function. *Arch Med Sci* 2010; **6**: 11-18 [PMID: 22371714 DOI: 10.5114/aoms.2010.13500]

63 **Didangelos T**, Moralidis E, Karlafti E, Tziomalos K, Margaritidis C, Kontoninas Z, Stergiou I, Boulbou M, Papagianni M, Papanastasiou E, Hatzitolios AI. A Comparative Assessment of Cardiovascular Autonomic Reflex Testing and Cardiac ^{123}I-Metaiodobenzylguanidine Imaging in Patients with Type 1 Diabetes Mellitus without Complications or Cardiovascular Risk Factors. *Int J Endocrinol* 2018; **2018**: 5607208 [PMID: 29721015 DOI: 10.1155/2018/5607208]

64 **Migisha R**, Agaba DC, Katamba G, Kwaga T, Tumwesigye R, Miranda SL, Muyingo A, Siedner MJ. Prevalence and Correlates of Cardiovascular Autonomic Neuropathy Among Patients with Diabetes in Uganda: A Hospital-Based Cross-sectional Study. *Glob Heart* 2020; **15**: 21 [PMID: 32489794 DOI: 10.5334/gh.765]

65 **Pafili K**, Trypsianis G, Papazoglou D, Maltezos E, Papanas N. Simplified Diagnosis of Cardiovascular Autonomic Neuropathy in Type 2 Diabetes Using Ewing's Battery. *Rev Diabet Stud* 2015; **12**: 213-219 [PMID: 26676669 DOI: 10.1900/RDS.2015.12.213]

66 **Travin MI**. Current Clinical Applications and Next Steps for Cardiac Innervation Imaging. *Curr Cardiol Rep* 2017; **19**: 1 [PMID: 28084596 DOI: 10.1007/s11886-017-0817-2]

67 **Tamura K**, Utsunomiya K, Nakatani Y, Saika Y, Onishi S, Iwasaka T. Use of iodine-123 metaiodobenzylguanidine scintigraphy to assess cardiac sympathetic denervation and the impact of hypertension in patients with non-insulin-dependent diabetes mellitus. *Eur J Nucl Med* 1999; **26**: 1310-1316 [PMID: 10541830 DOI: 10.1007/s002590050588]

68 **Kreiner G**, Wolzt M, Fasching P, Leitha T, Edlmayer A, Korn A, Waldhäusl W, Dudczak R. Myocardial m-[123I]iodobenzylguanidine scintigraphy for the assessment of adrenergic cardiac innervation in patients with IDDM. Comparison with cardiovascular reflex tests and relationship to left ventricular function. *Diabetes* 1995; **44**: 543-549 [PMID: 7729613 DOI: 10.2337/diab.44.5.543]

69 **Scholte AJ**, Schuijf JD, Delgado V, Kok JA, Bus MT, Maan AC, Stokkel MP, Kharagitsingh AV, Dibbets-Schneider P, van der Wall EE, Bax JJ. Cardiac autonomic neuropathy in patients with diabetes and no symptoms of coronary artery disease: comparison of 123I-metaiodobenzylguanidine myocardial scintigraphy and heart rate variability. *Eur J Nucl Med Mol Imaging* 2010; **37**: 1698-1705 [PMID: 20411258 DOI: 10.1007/s00259-010-1442-0]

70 **Scott LA**, Kench PL. Cardiac autonomic neuropathy in the diabetic patient: does 123I-MIBG imaging have a role to play in early diagnosis? *J Nucl Med Technol* 2004; **32**: 66-71 [PMID: 15175402]

71 **Travin MI**, Matsunari I, Thomas GS, Nakajima K, Yoshinaga K. How do we establish cardiac sympathetic nervous system imaging with ^{123}I-mIBG in clinical practice? *J Nucl Cardiol* 2019; **26**: 1434-1451 [PMID: 30178272 DOI: 10.1007/s12350-018-1394-5]

72 **Viggiano A**, Vicidomini C, Monda M, Carleo D, Carleo R, Messina G, Viggiano A, Viggiano E, De Luca B. Fast and low-cost analysis of heart rate variability reveals vegetative alterations in noncomplicated diabetic patients. *J Diabetes Complications* 2009; **23**: 119-123 [PMID: 18413209 DOI: 10.1016/j.jdiacomp.2007.11.009]

73 **Billman GE**. Heart rate variability - a historical perspective. *Front Physiol* 2011; **2**: 86 [PMID: 22144961 DOI: 10.3389/fphys.2011.00086]

74 **Vanderlei LC**, Pastre CM, Hoshi RA, Carvalho TD, Godoy MF. Basic notions of heart rate variability and its clinical applicability. *Rev Bras Cir Cardiovasc* 2009; **24**: 205-217 [PMID: 19768301 DOI: 10.1590/s0102-76382009000200018]

75 rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; **93**: 1043-1065 [PMID: 8598068 DOI: 10.1161/01.CIR.93.5.1043]

76 **McCraty R**, Shaffer F. Heart Rate Variability: New Perspectives on Physiological Mechanisms, Assessment of Self-regulatory Capacity, and Health risk. *Glob Adv Health Med* 2015; **4**: 46-61 [PMID: 25694852 DOI: 10.7453/gahmj.2014.073]

77 **Hillebrand S**, Gast KB, de Mutsert R, Swenne CA, Jukema JW, Middeldorp S, Rosendaal FR, Dekkers OM. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace* 2013; **15**: 742-749 [PMID: 23370966 DOI: 10.1093/europace/eus341]

78 **Farah BQ**. Heart Rate Variability as an Indicator of Cardiovascular Risk in Young Individuals. *Arq Bras Cardiol* 2020; **115**: 59-60 [PMID: 32785499 DOI: 10.36660/abc.20200444]

79 **Goldenberg I**, Goldkorn R, Shlomo N, Einhorn M, Levitan J, Kuperstein R, Klempfner R, Johnson B. Heart Rate Variability for Risk Assessment of Myocardial Ischemia in Patients Without Known Coronary Artery Disease: The HRV-DETECT (Heart Rate Variability for the Detection of Myocardial Ischemia) Study. *J Am Heart Assoc* 2019; **8**: e014540 [PMID: 31838969 DOI: 10.1161/JAHA.119.014540]

80 **Witzel II**, Jelinek HF, Khalaf K, Lee S, Khandoker AH, Alsafar H. Identifying Common Genetic Risk Factors of Diabetic Neuropathies. *Front Endocrinol (Lausanne)* 2015; **6**: 88 [PMID: 26074879 DOI: 10.3389/fendo.2015.00088]

81 **McCarty N**, Silverman B. Cardiovascular autonomic neuropathy. *Proc (Bayl Univ Med Cent)* 2016; **29**: 157-159 [PMID: 27034552 DOI: 10.1080/08998280.2016.11929397]

82 **Rolim LC**, de Souza JS, Dib SA. Tests for early diagnosis of cardiovascular autonomic neuropathy: critical analysis and relevance. *Front Endocrinol (Lausanne)* 2013; **4**: 173 [PMID: 24273533 DOI: 10.3389/fendo.2013.00173]

83 **Metelka R**, Cibičková L, Gajdová J, Krystyník O. Heart rate variability evaluation in the assessment of cardiac autonomic neuropathy in patients with type 2 diabetes. *Cor et Vasa* 2018; **60**: e335-e44 [DOI: 10.1016/j.crvasa.2017.05.001]

84 **Tarvainen MP**, Cornforth DJ, Kuoppa P, Lipponen JA, Jelinek HF. Complexity of heart rate variability in type 2 diabetes - effect of hyperglycemia. *Annu Int Conf IEEE Eng Med Biol Soc* 2013; **2013**: 5558-5561 [PMID: 24110996 DOI: 10.1109/EMBC.2013.6610809]

85 **Kempler P**, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, Kerényi Z, Tamás G, Ward JD, Fuller JH; EURODIAB IDDM Complications Study Group. Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM Complications Study. *Diabet Med* 2002; **19**: 900-909 [PMID: 12421426 DOI: 10.1046/j.1464-5491.2002.00821.x]

86 **Witte DR**, Tesfaye S, Chaturvedi N, Eaton SE, Kempler P, Fuller JH; EURODIAB Prospective Complications Study Group. Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. *Diabetologia* 2005; **48**: 164-171 [PMID: 15619072 DOI: 10.1007/s00125-004-1617-y]

87 **Ziegler D**, Zentai CP, Perz S, Rathmann W, Haastert B, Döring A, Meisinger C; KORA Study Group. Prediction of mortality using measures of cardiac autonomic dysfunction in the diabetic and nondiabetic population: the MONICA/KORA Augsburg Cohort Study. *Diabetes Care* 2008; **31**: 556-561 [PMID: 18086873 DOI: 10.2337/dc07-1615]

88 **Orlov S**, Cherney DZ, Pop-Busui R, Lovblom LE, Ficociello LH, Smiles AM, Warram JH, Krolewski AS, Perkins BA. Cardiac autonomic neuropathy and early progressive renal decline in patients with nonmacroalbuminuric type 1 diabetes. *Clin J Am Soc Nephrol* 2015; **10**: 1136-1144 [PMID: 26092828 DOI: 10.2215/CJN.11441114]

89 **Shaffer F**, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Health* 2017; **5**: 258 [PMID: 29034226 DOI: 10.3389/fpubh.2017.00258]

90 **Young HA**, Benton D. Heart-rate variability: a biomarker to study the influence of nutrition on physiological and psychological health? *Behav Pharmacol* 2018; **29**: 140-151 [PMID: 29543648 DOI: 10.1097/FBP.0000000000000383]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 868-882

DOI: 10.4239/wjd.v12.i6.868

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Estimated impact of introduction of new diagnostic criteria for gestational diabetes mellitus

Leon de Wit, Anna B Zijlmans, Doortje Rademaker, Christiana A Naaktgeboren, J Hans DeVries, Arie Franx, Rebecca C Painter, Bas B van Rijn

ORCID number: Leon de Wit 0000-0002-9159-8065; Anna B Zijlmans 0000-0003-4007-244X; Doortje Rademaker 0000-0002-3315-1804; Christiana A Naaktgeboren 0000-0002-0685-9989; J Hans DeVries 0000-0001-9196-9906; Arie Franx 0000-0001-8801-5546; Rebecca C Painter 0000-0001-9336-6033; Bas B van Rijn 0000-0003-1305-3825.

Author contributions: van Rijn BB contributed to the conception of the study and is the guarantor for the contents of the article, and as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the analyses performed; de Wit L and Zijlmans AB collected the data, performed the analyses, drafted the manuscript and coordinated revisions; Statistical support was provided by Naaktgeboren CA; All authors have contributed to the design of the study, performed interpretation of the data and critical revision of the article for important intellectual content and approved the final version of the manuscript for publication.

Institutional review board statement: This study was exempt from approval of the Medical Research Ethics Committee of the

Leon de Wit, Department of Obstetrics and Gynaecology, University Medical Center Utrecht, Utrecht 3584 EA, Netherlands

Anna B Zijlmans, Department of Obstetrics and Gynaecology, Gelderse Vallei Hospital, Ede 6716 RP, Netherlands

Doortje Rademaker, Department of Obstetrics and Gynaecology, Amsterdam University Medical Centers- Location AMC, Amsterdam 1105 AZ, Netherlands

Christiana A Naaktgeboren, Rebecca C Painter, Department of Obstetrics and Gynaecology, Amsterdam University Medical Centers–Location AMC, Amsterdam 1105 AZ, Netherlands

J Hans DeVries, Department of Endocrinology, Amsterdam University Medical Centers–Location AMC, Amsterdam 1105 AZ, Netherlands

Arie Franx, Bas B van Rijn, Department of Obstetrics and Fetal Medicine, Erasmus MC Sophia Children Hospital, Rotterdam 3015 GD, Netherlands

Corresponding author: Bas B van Rijn, MD, MSc, PhD, Assistant Professor, Department of Obstetrics and Fetal Medicine, Erasmus MC Sophia Children Hospital, Doctor Molewaterplein 40, Rotterdam 3015 GD, Netherlands. b.vanrijn@erasmusmc.nl

Abstract

BACKGROUND

Implementation of new diagnostic criteria for gestational diabetes mellitus (GDM) are still a subject of debate, mostly due to concerns regarding the effects on the number of women diagnosed with GDM and the risk profile of the women newly diagnosed.

AIM

To estimate the impact of the World Health Organization (WHO) 2013 criteria compared with the WHO 1999 criteria on the incidence of gestational diabetes mellitus as well as to determine the diagnostic accuracy for detecting adverse pregnancy outcomes.

METHODS

We retrospectively analyzed a single center Dutch cohort of 3338 women

University Medical Center Utrecht (reference number 16-711/C), which granted a waiver after reviewing the protocol because the Dutch Medical Research Involving Human Subjects Act (WMO) did not apply to this study.

Informed consent statement: With this document we would like to inform you that for the manuscript regarding our study entitled 'Estimated impact of introduction of new diagnostic criteria for gestational diabetes mellitus' no individual informed consent forms have been signed by participants as approved by our Institutional Review Board (MREC of the University Medical Center Utrecht). See the attached Institutional Review Board Approval Form.

Conflict-of-interest statement: The authors declare that there is no duality of interest associated with this manuscript.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

undergoing a 75 g oral glucose tolerance test where the WHO 1999 criteria to diagnose GDM were clinically applied. Women were categorized into four groups: non-GDM by both criteria, GDM by WHO 1999 only (excluded from GDM), GDM by WHO 2013 only (newly diagnosed) and GDM by both criteria. We compared maternal characteristics, pregnancy outcomes and likelihood ratios for adverse pregnancy outcomes.

RESULTS

Retrospectively applying the WHO 2013 criteria increased the cohort incidence by 13.1%, from 19.3% to 32.4%. Discordant diagnoses occurred in 21.3%; 4.1% would no longer be labelled as GDM, and 17.2% were newly diagnosed. Compared to the non-GDM group, women newly diagnosed were older, had higher rates of obesity, higher diastolic blood pressure and higher rates of caesarean deliveries. Their infants were more often delivered preterm, large-for-gestational-age and were at higher risk of a 5 min Apgar score < 7. Women excluded from GDM were older and had similar pregnancy outcomes compared to the non-GDM group, except for higher rates of shoulder dystocia (4.3% *vs* 1.3%, P = 0.015). Positive likelihood ratios for adverse outcomes in all groups were generally low, ranging from 0.54 to 2.95.

CONCLUSION

Applying the WHO 2013 criteria would result in a substantial increase in GDM diagnoses. Newly diagnosed women are at increased risk for pregnancy adverse outcomes. This risk, however, seems to be lower than those identified by the WHO 1999 criteria. This could potentially influence the treatment effect that can be achieved in this group. Evidence on treatment effects in newly diagnosed women is urgently needed.

Key Words: Diagnostic criteria; Gestational diabetes; Glucose tolerance test; Incidence; Pregnancy outcome

Core Tip: The World Health Organization 2013 criteria would increase the number of women diagnosed with gestational diabetes mellitus to almost one third of the population tested. Our data confirm that the new criteria indeed identify women at risk, implying potential for treatment. However, we also show that implementation of the criteria would result in a great increase of women diagnosed with gestational diabetes mellitus, resulting in over half of the women to be subjected to unevaluated treatment, as evidenced by the treatment effect in this group is currently absent. This stresses the need for randomized trials to evaluate the new criteria prior to implementation.

Citation: de Wit L, Zijlmans AB, Rademaker D, Naaktgeboren CA, DeVries JH, Franx A, Painter RC, van Rijn BB. Estimated impact of introduction of new diagnostic criteria for gestational diabetes mellitus. *World J Diabetes* 2021; 12(6): 868-882
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/868.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.868

INTRODUCTION

Gestational diabetes mellitus (GDM), defined as hyperglycemia first diagnosed in pregnancy that is not overt diabetes mellitus, is an increasing health problem worldwide and associated with substantial adverse maternal and fetal effects[1]. Adequate recognition, diagnosis and treatment of GDM improves health outcomes in mothers and their offspring[2-5]. Diagnostic criteria for GDM vary globally and are still much debated[6-9]. Based on the 2008 Hyperglycemia and Adverse Pregnancy Outcome study, new criteria were developed by the International Association of Diabetes in Pregnancy Study Groups, which were adopted by the World Health Organization (WHO) in 2013[1,10,11]. Uniform implementation of the revised criteria

Country/Territory of origin: Netherlands

Peer-review report's scientific quality classification

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

Received: February 2, 2021
Peer-review started: February 2, 2021
First decision: February 25, 2021
Revised: March 12, 2021
Accepted: April 25, 2021
Article in press: April 25, 2021
Published online: June 15, 2021

P-Reviewer: Ling ZX
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Ma YJ

is currently held back due to ongoing concerns regarding their impact on prevalence and associated health care costs[12,13]. Previous studies have shown a substantial absolute increase of GDM prevalence ranging from 0.9% to as much as 25.9%, following conversion from the 1999 to the 2013 criteria, with limited data on their direct effect on pregnancy outcomes[12,14-28].

The impact of the widening of disease definitions, as is the case with the proposed changes in diagnostic criteria for GDM, has gained attention due to concerns regarding possible overdiagnosis, medicalization and overtreatment[12,13]. The WHO 2013 criteria propose a lower fasting, a higher post load glucose value and an additional one hour post load glucose value compared to the 1999 criteria. This leads to a newly diagnosed group of women based on their fasting glucose and a group of women no longer qualifying for the diagnosis based on their two-hour glucose value. Apart from an overall expected increase of the incidence of GDM as a result of the new criteria, the composition of the group of women labeled as GDM is therefore also likely to change. This is of interest in the assessment of incremental health benefits, potential harm and cost-effectiveness, which must be balanced for both individual patients and society prior to introduction of new criteria.

The aim of this study was to assess the potential impact of adopting the WHO 2013 criteria on the incidence of GDM. We sought to investigate the differences in patient characteristics of women with a discordant diagnosis of GDM between criteria, whether pregnancy outcomes in the newly diagnosed group differ from the nondiabetic population and to study the diagnostic value of the different criteria for adverse pregnancy outcomes.

MATERIALS AND METHODS

Setting and study population

We conducted a retrospective cohort study including all pregnant women at risk for GDM, booked for an oral glucose tolerance test (OGTT) and receiving obstetric care in the University Medical Center Utrecht from 1 August 2011–27 October 2016. This study was exempt from approval of the Medical Research Ethics Committee of the University Medical Center Utrecht (reference number 16-711/C), which granted a waiver after reviewing the protocol because the Dutch Medical Research Involving Human Subjects Act did not apply to this study. Therefore, no individual informed consent was required for this study. Data was anonymized and collectively analyzed to ensure there was no risk of disclosure of the identity of included subjects.

Women were identified using laboratory data, which were cross-referenced with obstetric patient files. Data was collected *per* pregnancy, meaning that women with OGTTs in multiple pregnancies during the study period were included as separate cases. Women undergoing an OGTT for another reason than to test for GDM were excluded.

Screening for GDM

Pregnant women with one or more predefined risk factors underwent screening between 24 and 28 wk of gestation by means of a 2-point 75 g OGTT[29]. Risk factors following Dutch national guidelines that prompted screening included a history of GDM, body mass index (BMI) > 30 kg/m^2 at intake, previous delivery of a child with a birth weight > 95th centile or > 4500 g, a first-degree family member with diabetes, ethnic predisposition, history of unexplained stillbirth or polycystic ovarian syndrome [29]. Those with a history of GDM underwent screening at 16 wk of gestation, which was repeated at 24-28 wk if initially normal. Finally, women with clinical signs suggestive for GDM (*e.g.*, polyhydramnios, suspected fetal macrosomia or polydipsia) could undergo OGTT testing at any gestational age.

Classification of OGTT results

Based on the OGTT results, we retrospectively classified the women into four groups: (1) no GDM according to both the WHO 1999 and WHO 2013 criteria: fasting glucose < 5.1 mmol/L and 2-h post load glucose < 7.8 mmol/L; (2) GDM according to the WHO 1999 criteria but not the WHO 2013 criteria: fasting glucose < 5.1 mmol/L and 2-h post load glucose ≥ 7.8 mmol/L but < 8.5 mmol/L; (3) GDM according to the WHO 2013 criteria but not the WHO 1999 criteria: fasting glucose ≥ 5.1 mmol/L but < 7.0 mmol/L and 2-h post load glucose < 7.8 mmol/L; and (4) GDM according to both criteria: either fasting glucose ≥ 7.0 mmol/L and/or 2-h post load glucose ≥ 8.5 mmol/L alone, or a combination of fasting glucose ≥ 5.1 mmol/L and 2-h post load glucose ≥ 7.8

mmol/L.

Groups 1 and 4 were groups where both the assessed criteria agree, and in groups 2 and 3 there is a discordance in diagnosis. For this study, group 1 was labelled non-GDM. Group 2 and 4 received treatment for GDM. Group 2 represents the group of women who would no longer be labelled as GDM with the new criteria, and group 3 consists of the newly added women that would switch from non-GDM to GDM. Some women had received more than one OGTT in a single pregnancy, for instance due to newly suspected macrosomia occurring after an initial negative routine screening. Results from all OGTTs were considered when classifying women into group 1 to 4 (Supplemental table 1). The 1-h post load glucose threshold of ≥ 10.0 mmol/L of the WHO 2013 criteria was not used in the classification, as these results were not available.

Routine care for GDM

Women started treatment if diagnosed with GDM according to the WHO 1999 criteria, comprising all women in groups 2 and 4. They received personalized dietary advice and were instructed to self-monitor fasting and postprandial glucose concentrations by finger stick at least twice a week. Target glucose values were fasting ≤ 5.3 mmol/L and 2-h postprandial ≤ 6.7 mmol/L. In case of insufficient glycemic regulation after 1 wk to 2 wk of dietary intervention, insulin treatment was initiated. Women with normal OGTT results according to the WHO 1999 criteria, *e.g.*, groups 1 and 3, received care as usual.

Data collection

OGTT results, patient characteristics and pregnancy outcomes were collected from electronic patient files. Given that women without risk factors are not screened for GDM and that OGTT screening also took place in laboratories outside the University Medical Center Utrecht, we were not able to provide a population or regional incidence estimate of GDM but rather a cohort incidence.

Patient characteristics and outcomes

We calculated BMI using prepregnancy reported height and weight. Regarding ethnicity, Hindustan was used for women from the South Asian/Indian subcontinent or Surinamese descent. Mediterranean ethnicity comprised mostly women originating from Turkey and Morocco. Small-for-gestational-age (SGA) and large-for-gestational-age (LGA) were defined as neonatal birth weight < 10^{th} and > 90^{th} percentile for gestational age, respectively, using the Dutch reference curves[30]. Preterm birth was defined as a gestational age at birth < 37 wk, which was further divided in either spontaneous or indicated preterm birth. Individual diagnoses of gestational hypertension and preeclampsia were not available. As a proxy for hypertensive disorders during the pregnancy, the highest measured diastolic blood pressure was used for analysis.

Statistical analysis and reporting of data

The statistical methods of this study were reviewed by Christiana Naaktgeboren from the Department of Obstetrics and Gynaecology, Amsterdam University Medical Centers–Location AMC. GDM incidence in the cohort was calculated by applying both the WHO 1999 and 2013 criteria. We produced 2 × 2 contingency tables to display the number of women in groups 1 through 4, based on their OGTT results. Patient characteristics and pregnancy outcomes were reported and analyzed using groups 1 to 4 as determinants. Continuous variables were reported as mean ± SD or as median and interquartile range depending on distribution. Number and percentage were reported for categorical variables. Differences between groups were assessed using group 1 (non-GDM) as the reference group. We compared continuous variables using the independent t-test or Mann-Whitney U test and the χ-square test or Fisher's exact test for categorical data. Furthermore, we assessed the diagnostic value of the OGTT by calculating positive and negative likelihood ratios for adverse obstetric and neonatal outcomes for groups 2 to 4, using group 1 as the reference group[31,32]. A P value < 0.05 was considered statistically significant. Analyses were performed using SPSS 25.0 for Windows (IBM SPSS, Chicago, IL, United States).

RESULTS

In the study period 3628 women were scheduled for an OGTT. Of these, 290 were excluded: 67 were planned for an OGTT but did not undergo the test and another 223 were excluded because they underwent an OGTT for other reasons than to test for GDM. In total we included 3338 women for analysis (Figure 1).

Effect of WHO 2013 criteria on GDM incidence

Of the 3338 included women, 643 (19.3%) were diagnosed with GDM using the WHO 1999 criteria. Retrospectively applying the WHO 2013 criteria resulted in 1082 women diagnosed with GDM, corresponding to a cohort incidence of 32.4% (Figure 1). This is equivalent to a relative increase of the incidence of 68%. A total of 2219 women (63.5%) had normal glucose tolerance according to both criteria (group 1), 506 women (15.2%) were diagnosed with GDM by both criteria (group 4), 137 women (4.1%) were diagnosed by WHO 1999 criteria only (group 2) and 576 (17.2%) by the WHO 2013 criteria only (group 3) (Figure 1).

Maternal characteristics

Maternal characteristics are presented in Table 1. Compared to group 1 (non-GDM) women in group 3 (newly added by the WHO 2013 criteria) were older (33.0 *vs* 32.3 years, $P = 0.002$) and had a higher median prepregnancy BMI (29.0 kg/m^2 *vs* 24.3 kg/m^2, $P < 0.001$). BMI distribution was significantly different ($P < 0.001$), with a higher frequency of BMIs of 30-35 and ≥ 35 kg/m^2 in the newly added group compared to the non-GDM women (24.4% *vs* 10.4% and 19.0% *vs* 5.9%, respectively). There were no differences in ethnicity between the groups. In group 3 smoking during pregnancy was reported more often (16.8% *vs* 13.3%, $P = 0.031$), and women were less often primiparous (29.7% *vs* 37.9%, $P < 0.001$).

The women in group 2 (GDM according to the WHO 1999 but not the WHO 2013 criteria) had a higher median age compared to the non-GDM group (34.1 *vs* 32.3 years, $P < 0.001$) and more often were ≥ 35 years of age (40.1% *vs* 29.0%, $P = 0.002$). Median BMI and BMI distribution as well as other baseline characteristics were similar to the non-GDM group.

The women in group 4 (GDM according to both criteria) had a significantly higher median age, were more often ≥ 35 years of age, had a higher prepregnancy BMI and had higher rates of obesity compared to the non-GDM women. In this group Caucasian ethnicity was less frequent, while Asian ethnicity was more frequent.

Pregnancy outcomes

Maternal and neonatal outcomes *per* group are presented in Table 2. Compared to the non-GDM group, women in group 3 were more likely to have a highest diastolic blood pressure ≥ 90 mmHg (18.6% *vs* 13.9%, $P = 0.007$) and had similar rates of induction of labor, assisted vaginal deliveries and emergency caesarean section but were more likely to deliver by planned caesarean section (15.5% *vs* 11.1%, $P = 0.006$). Indicated preterm birth occurred more often (1.2% *vs* 0.3%, $P = 0.024$). Also, women in group 3 gave birth to children with higher median birth weight (3598 *vs* 3490 g, $P < 0.001$), had higher rates of birth weight > 4000 g (22.3% *vs* 16.2%, $P < 0.001$), LGA (16.2% *vs* 10.2%, $P < 0.001$) and higher rates of 5-min Apgar score < 7 (3.9% *vs* 2.0%, $P = 0.01$) compared to the offspring of women in the reference group 1 with normal glucose tolerance. SGA occurred less frequently in group 3 (5.4% *vs* 8.0%, $P < 0.001$).

In group 2, labor was more often induced (28.8% *vs* 17.4%, $P = 0.001$) compared to the non-GDM group, as this is recommended in the Dutch guidelines for GDM. Rates of birth weight > 4000 g, LGA and SGA were comparable between group 2 (15.0%, 12.2% and 4.3%) and group 1 (all $P > 0.05$). Other neonatal outcomes were also similar, except for shoulder dystocia, which occurred in 4.3% compared to 1.3% in group 1 ($P = 0.015$).

Women in group 4 were more likely to have a highest recorded diastolic blood pressure ≥ 90 mmHg, labor was induced more often and planned caesarean section rates were higher compared to the non-GDM group. Assisted vaginal deliveries were carried out less frequently in this group (3.6% *vs* 6.4%, $P = 0.018$). Rates of both spontaneous and indicated preterm birth were also significantly higher (6.9% *vs* 4.1%, $P = 0.008$ and 1.5% *vs* 0.3%, $P = 0.006$ respectively) compared to group 1. Birth weight > 4000 g and SGA did not differ between group 4 and the reference group 1. However, more infants were LGA (16.6% *vs* 10.2%, $P < 0.001$). Also, neonates were more likely to be admitted to the neonatology ward, and shoulder dystocia and a 5-min Apgar score < 7 occurred more often.

Table 1 Patient characteristics *per* group based on classification of oral glucose tolerance test results

	n (%)	Group 1: non-GDM	Group 2: GDM by WHO 1999 only[1]	*P* value[2]	Group 3: GDM by WHO 2013 only	*P* value[2]	Group 4: GDM by both criteria[1]	*P* value[2]
n (%)	3338	2119 (63.5)	137 (4.1)		576 (17.2)		506 (15.2)	
Age, yr	3336	32.3 (28.5-35.7)	34.1 (30.4-38.0)	< 0.001	33.0 (29.0-36.7)	0.002	33.6 (30.3-37.0)	< 0.001
Age group, *n* (%)	3336							
< 30 yr		698 (33.0)	31 (22.6)	0.002	174 (30.2)	0.031	117 (23.1)	< 0.001
30-35 yr		806 (38.1)	51 (37.2)		204 (35.4)		185 (36.6)	
≥ 35 yr		613 (29.0)	55 (40.1)		198 (34.4)		204 (40.3)	
Prepregnancy BMI, kg/m^2	3156	24.3 (21.8-27.9)	24.7 (22.7-28.7)	0.072	29.0 (24.5-33.6)	< 0.001	28.0 (24.2-32.3)	< 0.001
Prepregnancy BMI group, *n* (%)	3156							
< 30 kg/m^2		1669 (83.7)	104 (80.6)	0.598	309 (56.6)	< 0.001	314 (64.5)	< 0.001
≥ 30-< 35 kg/m^2		207 (10.4)	18 (14.0)		133 (24.4)		102 (20.9)	
≥ 35 kg/m^2		118 (5.9)	7 (5.4)		104 (19.0)		71 (14.6)	
Ethnicity, *n* (%)	3248							
Caucasian		1126 (54.8)	69 (51.1)	0.402	292 (51.7)	0.378	241 (48.7)	0.030
Mediterranean		616 (30.0)	40 (29.6)		176 (31.2)		166 (33.5)	
African, Caribbean		72 (3.5)	3 (2.2)		19 (3.4)		17 (3.4)	
Asian		53 (2.6)	7 (5.2)		16 (2.8)		22 (4.4)	
Hindustan		37 (1.8)	3 (2.2)		18 (3.2)		15 (3.0)	
Other		150 (7.1)	13 (9.5)		44 (7.6)		34 (6.7)	
Gravidity	3336	2 (1-3)	2 (1-3)	0.368	2 (2-4)	< 0.001	2 (2-3)	0.045
Parity, *n* (%)	3323							
0		803 (37.9)	46 (33.6)	0.102	171 (29.7)	< 0.001	169 (33.4)	0.010
1		785 (37.0)	48 (35.0)		231 (40.1)		183 (36.2)	
≥ 2		519 (24.9)	43 (31.4)		173 (30.0)		152 (30.0)	
Smoking during pregnancy, *n* (%)	3270	277 (13.3)	15 (11.1)	0.470	94 (16.8)	0.031	79 (16.1)	0.104
Conception spontaneous, *n* (%)	3277	1866 (89.8)	120 (88.9)	0.748	496 (87.9)	0.216	431 (86.4)	0.029
Multiple pregnancy, *n* (%)	3336	84 (4.0)	9 (6.6)	0.138	10 (1.7)	0.010	33 (6.5)	0.012
Gestational age at OGTT, wk	3336	27.1 (25.1-29.6)	28.1 (25.4-32.7)	0.041	27.0 (24.6-29.1)	< 0.001	27.0 (23.9-29.7)	< 0.001
OGTT fasting plasma glucose value, mmol/L	3336	4.6 (4.4-4.8)	4.7 (4.5-4.8)	0.087	5.2 (5.1-5.4)	< 0.001	5.4 (5.0-5.8)	< 0.001
OGTT 2-h post load glucose value, mmol/L	3298	5.7 (4.9-6.4)	8.0 (7.8-8.2)	< 0.001	6.4 (5.7-6.9)	< 0.001	8.7 (8.1-9.6)	< 0.001

Continuous variables are presented as mean ± SD or median (interquartile range). Percentages may not add up to 100% due to rounding.
[1]Women in this group received treatment for gestational diabetes mellitus.
[2]*P* value reported compared to group 1: non-gestational diabetes mellitus.
GDM: Gestational diabetes mellitus; BMI: Body mass index; OGTT: Oral glucose tolerance test; WHO: World Health Organization.

Positive and negative likelihood ratios for adverse pregnancy outcomes with corresponding 95% confidence intervals *per* group are presented in Table 3. Compared to the non-GDM group, positive likelihood ratios (LR+) for adverse outcomes were generally higher in women with a positive OGTT according to either the WHO 1999, WHO 2013 or both criteria, except for assisted vaginal delivery and SGA. The LR+ for adverse

Table 2 Pregnancy outcomes *per* group based on classification of oral glucose tolerance test results

	n	Group 1: non-GDM	Group 2: GDM by WHO 1999 only[1]	*P* value[2]	Group 3: GDM by WHO 2013 only	*P* value[2]	Group 4: GDM by both criteria[1]	*P* value[2]
n (%)	3338	2119 (63.5)	137 (4.1)		576 (17.2)		506 (15.2)	
Maternal								
Highest diastolic blood pressure, mmHg	3103	75 (70-82)	80 (70-85)	0.060	80 (75-85)	< 0.001	80 (75-85)	< 0.001
Highest diastolic blood pressure, *n* (%)	3103							
< 90 mmHg		1691 (86.1)	111 (84.1)	0.520	429 (81.4)	0.007	379 (79.0)	< 0.001
≥ 90 mmHg		273 (13.9)	21 (15.9)	0.520	98 (18.6)	0.007	101 (21.0)	< 0.001
≥ 110 mmHg		31 (1.6)	3 (2.3)	0.541	4 (0.8)	0.156	7 (1.5)	0.849
Induction of labor, *n* (%)	3044	335 (17.4)	38 (28.8)	0.001	97 (19.0)	0.390	155 (32.6)	< 0.001
Planned caesarean, *n* (%)	3044	213 (11.1)	17 (12.9)	0.520	79 (15.5)	0.006	81 (17.1)	< 0.001
Assisted vaginal delivery, *n* (%)	3059	124 (6.4)	9 (6.8)	0.853	29 (5.6)	0.517	17 (3.6)	0.018
Emergency caesarean, *n* (%)	3059	223 (11.5)	12 (9.1)	0.394	65 (12.6)	0.492	65 (13.6)	0.205
Blood loss > 1000 cc, *n* (%)	3019	173 (9.1)	15 (11.4)	0.374	35 (6.9)	0.131	41 (8.7)	0.813
Neonatal								
Gestational age at delivery, wk	3070	40.0 (39.0-40.9)	39.4 (38.3-40.6)	0.001	39.9 (38.7-41.0)	0.515	39.1 (38.1-40.0)	< 0.001
Preterm birth, *n* (%)	3070							
Spontaneous		79 (4.1)	7 (5.3)	0.491	26 (5.0)	0.337	33 (6.9)	0.008
Indicated		6 (0.3)	1 (0.8)	0.370	6 (1.2)	0.024	7 (1.5)	0.006
Birth weight, grams	3186	3490 (3090-3840)	3449 (3041-3839)	0.654	3598 (3216-3943)	< 0.001	3420 (2969-3800)	0.056
Birth weight, percentile	3165	50.0 (24.8-75.8)	52.0 (28.8-76.4)	0.388	59.9 (32.6-81.6)	< 0.001	58.7 (29.5-82.1)	< 0.001
Birth weight > 4000 grams, *n* (%)	3186	325 (16.2)	21 (15.0)	0.719	117 (22.3)	< 0.001	81 (15.9)	0.896
Small-for-gestational-age (< 10th percentile), *n* (%)	3165	160 (8.0)	6 (4.3)	0.117	28 (5.4)	0.043	34 (6.7)	0.336
Large-for-gestational-age (> 90th percentile), *n* (%)	3165	203 (10.2)	17 (12.2)	0.435	84 (16.2)	< 0.001	84 (16.6)	< 0.001
Admission to neonatology ward, *n* (%)	3146	219 (11.0)	15 (10.7)	0.904	59 (11.4)	0.813	103 (20.4)	< 0.001
Admission to NICU, *n* (%)	3146	75 (3.8)	4 (2.9)	0.576	22 (4.3)	0.620	28 (5.5)	0.077
Shoulder dystocia, *n* (%)	3173	26 (1.3)	6 (4.3)	0.015	7 (1.3)	0.935	18 (3.5)	0.001
Apgar score 5 min < 7, *n* (%)	3155	39 (2.0)	4 (2.9)	0.525	20 (3.9)	0.010	24 (4.7)	< 0.001

Continuous variables are presented as mean ± SD or median (interquartile range). Percentages may not add up to 100% due to rounding.

[1]Women in this group received treatment for gestational diabetes mellitus.

[2]*P* value reported compared to group 1: non-gestational diabetes mellitus.

GDM: Gestational diabetes mellitus; NICU: Neonatal intensive care unit; WHO: World Health Organization.

outcome, excluding assisted vaginal delivery and SGA, ranged from 0.76 to 2.95 for all groups. An OGTT indicative for GDM according to both criteria (group 4) had the highest LR+ for most adverse outcomes. A positive OGTT by the WHO 2013 criteria only (group 3) had higher LR+ for adverse outcomes compared to a positive OGTT by WHO 1999 criteria only (group 2). Negative likelihood ratios showed a similar but inverse pattern that were mostly close to 1.00.

Table 3 Likelihood ratios for adverse pregnancy outcomes for group 2-4 compared to group 1

Outcome	*n* (%)	LR+	95%CI	LR-	95%CI
Highest diastolic BP ≥ 90 mmHg					
Non-GDM	273 (13.9)				
WHO 1999 criteria only	21 (15.9)	1.16	0.74-1.82	0.99	0.96-1.02
WHO 2013 criteria only	98 (18.6)	1.31	1.08-1.58	0.92	0.86-0.98
Both criteria	101 (21.0)	1.47	1.22-1.78	0.89	0.84-0.95
Planned caesarean					
Non-GDM	213 (11.1)				
WHO 1999 criteria only	17 (12.9)	1.18	0.72-1.92	0.99	0.95-1.03
WHO 2013 criteria only	79 (15.5)	1.35	1.10-1.66	0.91	0.85-0.98
Both criteria	81 (17.1)	1.47	1.20-1.81	0.89	0.83-0.96
Assisted vaginal delivery					
Non-GDM	124 (6.4)				
WHO 1999 criteria only	9 (6.8)	1.06	0.55-2.05	1.00	0.95-1.04
WHO 2013 criteria only	29 (5.6)	0.90	0.64-1.26	1.03	0.95-1.11
Both criteria	17 (3.6)	0.59	0.38-0.93	1.10	1.03-1.18
Emergency caesarean					
Non-GDM	223 (11.5)				
WHO 1999 criteria only	12 (9.1)	0.78	0.44-1.39	1.02	0.98-1.05
WHO 2013 criteria only	65 (12.6)	1.09	0.86-1.37	0.98	0.91-1.04
Both criteria	65 (13.6)	1.16	0.92-1.46	0.96	0.90-1.03
Preterm birth					
Non-GDM	87 (4.5)				
WHO 1999 criteria only	8 (6.1)	1.35	0.68-2.67	0.98	0.92-1.04
WHO 2013 criteria only	32 (6.2)	1.30	0.96-1.77	0.92	0.82-1.03
Both criteria	41 (8.6)	1.68	1.29-2.19	0.84	0.74-0.95
SGA					
Non-GDM	160 (8.0)				
WHO 1999 criteria only	6 (4.3)	0.54	0.24-1.20	1.03	1.00-1.07
WHO 2013 criteria only	28 (5.4)	0.71	0.50-1.00	1.08	1.01-1.15
Both criteria	34 (6.7)	0.86	0.63-1.18	1.04	0.97-1.11
Birth weight > 4000 g					
Non-GDM	325 (16.2)				
WHO 1999 criteria only	21 (15.0)	0.92	0.59-1.44	1.01	0.98-1.04
WHO 2013 criteria only	117 (22.3)	1.36	1.14-1.63	0.91	0.86-0.97
Both criteria	81 (15.9)	0.98	0.80-1.22	1.00	0.95-1.06
LGA					
Non-GDM	203 (10.2)				
WHO 1999 criteria only	17 (12.2)	1.22	0.75-1.98	0.99	0.95-1.03
WHO 2013 criteria only	84 (16.2)	1.50	1.23-1.83	0.88	0.81-0.95
Both criteria	84 (16.6)	1.54	1.26-1.87	0.87	0.81-0.94
Admission to NICU					

Non-GDM	75 (3.8)				
WHO 1999 criteria only	4 (2.9)	0.76	0.29-2.00	1.02	0.97-1.07
WHO 2013 criteria only	22 (4.3)	1.10	0.76-1.60	0.97	0.87-1.09
Both criteria	28 (5.5)	1.36	0.98-1.88	0.91	0.81-1.03
Shoulder dystocia					
Non-GDM	26 (1.3)				
WHO 1999 criteria only	6 (4.3)	2.95	1.41-6.19	0.87	0.73-1.03
WHO 2013 criteria only	7 (1.3)	1.03	0.53-1.99	0.99	0.83-1.19
Both criteria	18 (3.5)	2.06	1.43-2.96	0.74	0.58-0.94
5-min Apgar score < 7					
Non-GDM	39 (2.0)				
WHO 1999 criteria only	4 (2.9)	1.43	0.55-3.68	0.97	0.88-1.07
WHO 2013 criteria only	20 (3.9)	1.67	1.16-2.41	0.83	0.69-1.00
Both criteria	24 (4.7)	1.92	1.39-2.66	0.77	0.64-0.94

LR+: Positive likelihood ratio; 95%CI: 95% confidence interval; LR-: Negative likelihood ratio; GDM: Gestational diabetes mellitus; BP: Blood pressure; SGA: Small-for-gestational-age; LGA: Large-for-gestational-age; WHO: World Health Organization; NICU: Neonatal intensive care unit.

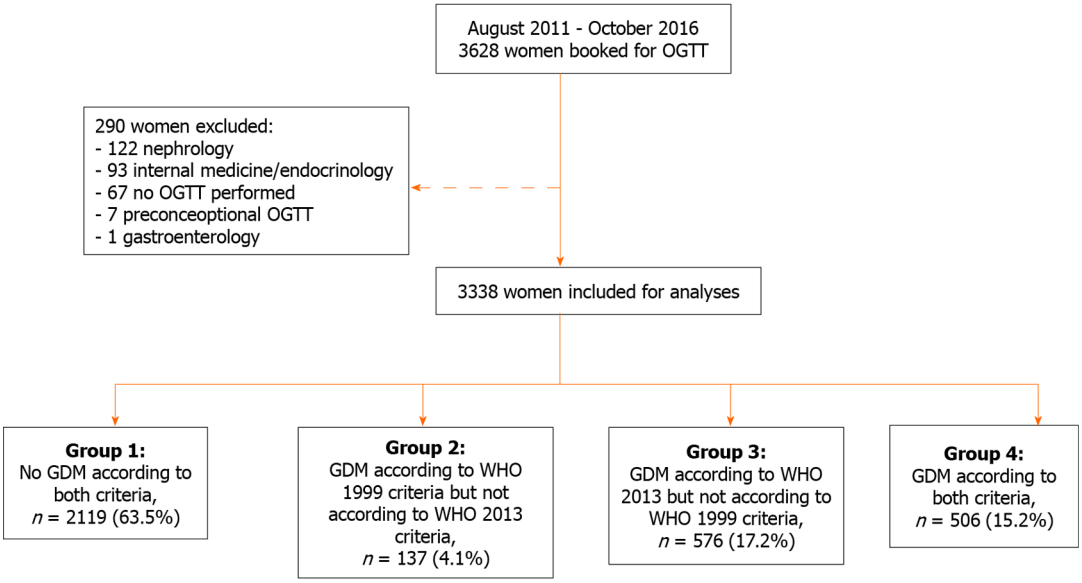

Figure 1 Cohort selection and flowchart of inclusions. OGTT: Oral glucose tolerance test; GDM: Gestational diabetes mellitus; WHO: World Health Organization.

DISCUSSION

In this study, we estimated the impact of adopting new criteria for GDM in a population where adherence to the traditional criteria is the current policy, with the aim to provide insight in the potential impact of modification of disease definitions for GDM. We showed that applying the WHO 2013 criteria would result in an absolute increase of GDM diagnoses in women at risk of 13.1% or a 1.7-fold increase in comparison to the current WHO 1999 criteria. The women newly identified by the WHO 2013 criteria showed less favorable patient characteristics, *i.e.* higher maternal age and BMI. Newly identified women also had an increased chance of adverse pregnancy outcomes, including higher blood pressure and higher rates of indicated preterm birth, LGA and 5-min Apgar score < 7, compared to the women with a normal glucose tolerance after screening. Women only diagnosed by the 1999 criteria had similar patient characteristics and obstetric outcomes compared to those with a normal OGTT, although the latter may be because they underwent GDM treatment.

An increase in the number of GDM diagnoses, as we found in our study, is in line with the original Hyperglycemia and Adverse Pregnancy Outcome findings, which were the trigger to expand the GDM definition[10]. However, effects of implementation of the WHO 2013 criteria show regional variation, depending on the population, screening approaches or the diagnostic criteria, which are in use prior to implementation[25-28,33,34]. Studies performed in Asian populations have reported both a decrease and increase in the number of GDM diagnoses[35-37]. The 1.7-fold increase in our cohort is similar to estimations from three previous European studies with similar risk-based screening strategies[16,19,38]. Others have reported an even greater increase after actual implementation of the WHO 2013 criteria, such as a 3.5-fold increase in a study from Spain and a 4-fold increase in a Swiss cohort[39,40]. In the United Arab Emirates, introduction of the new criteria would result in almost half of the pregnant population to be labelled as GDM[14].

In 2017 a multidisciplinary working group, which included members from the Guidelines International Network, Grading of Recommendations Assessment, Development and Evaluation working group and the WHO, proposed a checklist with issues that should be considered prior to introducing modified disease definitions[41]. Although the checklist has not been formally applied to the GDM definition expansion proposed by the WHO (2013), several points on the checklist have yet to be met. For example, evaluation of incremental benefits for patients classified by the new and the previous definition is needed. Our study complements a previous cohort study from the Netherlands, which similarly found that the women newly identified by the WHO 2013 criteria are at increased risk for adverse pregnancy outcomes compared to pregnant women with normal glucose tolerance[19]. Our analysis furthermore allowed us to directly compare the newly diagnosed group to those women in which the WHO 1999 criteria and WHO 2013 criteria agree, *e.g.*, expected 'severe' cases, and found that risks were generally lower in the newly added group. This is further strengthened by our analysis of likelihood ratios for adverse outcome for the different criteria. Positive likelihood ratios for adverse outcomes were lower in women with an abnormal OGTT by the WHO 2013 criteria compared to those positive by both criteria. However, an abnormal OGTT by any set of criteria showed only limited discriminative value in predicting adverse outcome, with the highest LR+ of 2.95 for shoulder dystocia and negative likelihood ratio mostly around 1.00.

Because the risk for adverse outcomes in the newly diagnosed women seems to be lower in comparison to the concordant group, it is this group of women facing possible overdiagnosis as a result of widening the diagnostic criteria for GDM. Multiple studies, all before-after studies, have shown positive effects on clinical outcomes, such as hypertensive disorders, LGA and caesarean section rates after implementation of the WHO 2013 criteria[39,42-44]. However, given the concurrent increase in the number of women diagnosed, it is unclear whether these results are attributable to effective treatment or due to inclusion of milder GDM cases. In one study that adjusted for maternal characteristics, only limited reductions in adverse outcomes were observed[42]. A randomized controlled trial found that treatment of milder GDM reduced LGA rates, shoulder dystocia, caesarean deliveries and hypertensive disorders[5]. The inclusion criteria for this trial do not fully correspond to the patients newly diagnosed by the WHO 2013 criteria, limiting extrapolation of these treatment results. To this date, no data are available for women with discordant diagnosis for GDM between the WHO 1999 and 2013 criteria and with that the treatment effect in this group specifically remains unknown. However, clinical trials on this matter are currently being undertaken[6,45,46].

Another point raised in the checklist is evaluation of potential incremental harm to patients. We found in our cohort that the proportion of women with an OGTT discordant between WHO 1999 and WHO 2013 was larger than the proportion of women in which both criteria agree. Implementation of the WHO 2013 criteria would subsequently result in unevaluated treatment of more than half the GDM population. This treatment could result in unnecessary exposure to interventions with possible harmful side effects, including induction of labor and caesarean sections[8]. Also, GDM diagnosis and subsequent medicalization can have profound negative effects on a patient's quality of life[47-51]. Similarly, underdiagnosis could occur in the group of women that are currently receiving treatment but would be excluded with the new criteria. Although this was a relatively small group in our cohort, evaluation is necessary to establish whether these women can be safely left untreated.

Other forms of incremental harm include increased costs and use of health care resources because of implementation of the new criteria[52]. With estimated costs for GDM treatment in excess of standard antenatal care of €6843 *per* individual[53], implementation of the new criteria would result in a direct increase of medical costs of

over 3 million euros for the study period in our center alone. In the Netherlands, approximately 5% of the pregnant population (approximately 8500 women) is diagnosed with GDM with the current criteria. A conservative 2% increase would result in 11900 diagnoses on a yearly basis, amounting up to over 23 million euros in additional health care costs. Data on cost-effectiveness are conflicting and are particularly influenced by the discriminative power of the new criteria to detect longer-term maternal, neonatal and infant outcome, which are currently largely unknown[39,54-56].

The strength of this study is the large sample size and availability of OGTT results, allowing for the reclassification in the four groups as presented in this study. This classification provides more insight on the impact of the WHO 2013 criteria by analyzing the women with discordant results separately, as proposed by the checklist on modifying disease definitions[41]. However, because the WHO 1999 criteria were used to diagnose GDM in our cohort, a treatment effect is present in the women meeting these criteria. Furthermore, the reference group in this study consisted of women with normal OGTT results. Because women were either screened because of the presence of risk factors or clinical signs suggestive for GDM, this group is a selection and therefore not fully representative of the general obstetric population in the Netherlands. However, comparison of pregnancy outcomes with a reference group including women without any risk factors for GDM would probably only further strengthen the associations we found. Another limitation is that there were no 1-h post load OGTT measurements available in this cohort, which are used in the WHO 2013 criteria. This may have resulted in an underestimation of the proportion of women diagnosed with GDM upon implementation of the WHO 2013 criteria.

CONCLUSION

The results from this retrospective study indicate a marked increase in the number of women diagnosed with GDM with the adoption of the WHO 2013 criteria as compared to WHO 1999 criteria. We have shown that the new criteria identify a new group of women at risk for adverse pregnancy outcomes but also result in exclusion of a number of women that currently receive treatment. Randomized trials are urgently needed to establish whether treatment of women with mild hyperglycemia, formerly not labelled as GDM, indeed leads to improvement of perinatal outcome. Treatment effects should be assessed both on the short-term and on the long-term of both mother and child to establish benefits, harms and cost-effectiveness of adopting the new criteria prior to implementation.

ARTICLE HIGHLIGHTS

Research background

Gestational diabetes mellitus (GDM) is the most common metabolic disorder of pregnancy. It is associated with both short- and long-term fetal, neonatal and maternal complications. Treatment of GDM has been shown to improve pregnancy outcomes.

Research motivation

Worldwide different diagnostic criteria to diagnose GDM are being used. Recently the Hyperglycemia and Adverse Pregnancy Outcome study has shown that maternal glucose levels below the most used thresholds increase the risk of adverse outcomes. As a result, new diagnostic criteria have been proposed by the World Health Organization (WHO) in 2013. These new, more stringent criteria have been shown to greatly affect the number of women diagnosed with GDM, which in turn can have great consequences for health care costs and effectiveness of current treatment strategies. However, the effects vary in different populations and are influenced by patient characteristics such as ethnicity and maternal body mass index.

Research objectives

We aimed to estimate the impact of the WHO 2013 criteria, compared with the WHO 1999 criteria, on the incidence of gestational diabetes mellitus as well as to determine the diagnostic accuracy for detecting adverse pregnancy outcomes. We sought to evaluate the patient characteristics and pregnancy outcomes of women with a discordant diagnosis specifically, as these are of importance for the treatment effects

that may be expected. Currently, the treatment effects in these women are unknown.

Research methods

For this study we evaluated a cohort of 3338 women that were tested for GDM using a 75 g oral glucose tolerance test in the University Medical Center Utrecht. We applied both the current WHO 1999 criteria and the newly proposed WHO 2013 criteria for GDM. We determined the change in the number of GDM diagnoses. Also, we separately reported on patient characteristics and pregnancy outcomes of women with discordant diagnoses and compared these to the non-GDM women. Lastly, we determined the likelihood ratios for adverse outcomes for the different groups.

Research results

Retrospectively applying the WHO 2013 criteria increased the cohort incidence by 13.1%, from 19.3 to 32.4%. Discordant diagnoses occurred in 21.3%; 4.1% would no longer be labelled as GDM, and 17.2% were newly diagnosed. Compared to the non-GDM group, women newly diagnosed were older, had higher rates of obesity, higher diastolic blood pressure and higher rates of caesarean deliveries. Their infants were more often delivered preterm, large-for-gestational-age and were at higher risk of a 5-min Apgar score < 7. Women excluded from GDM were older and had similar pregnancy outcomes compared to the non-GDM group, except for higher rates of shoulder dystocia (4.3% *vs* 1.3%, $P = 0.015$). Positive likelihood ratios for adverse outcomes in all groups were generally low, ranging from 0.54 to 2.95.

Research conclusions

The number of women diagnosed with GDM increases substantially with the WHO 2013 compared to the WHO 1999 criteria. Women additionally diagnosed are at increased risk for adverse pregnancy outcomes. However, they seem to be at lower risk than women who would be diagnosed with GDM by both the old and new criteria. Also, likelihood ratios for adverse outcomes comparing both diagnostic criteria are generally low. Treatment effects may therefore be lower in newly diagnosed women, which may result in overtreatment of women newly diagnosed with GDM according to the WHO 2013 criteria.

Research perspectives

Adopting the WHO 2013 criteria results in an increased number of women diagnosed with GDM and translates to an excess risk of adverse pregnancy outcomes, supporting the need for intervention studies to estimate the treatment benefit and cost-effectiveness to improve clinically relevant outcomes for these previously untreated pregnant women.

REFERENCES

1 criteria and classification of hyperglycaemia first detected in pregnancy: a World Health Organization Guideline. *Diabetes Res Clin Pract* 2014; **103**: 341-363 [PMID: 24847517 DOI: 10.1016/j.diabres.2013.10.012]

2 **Crowther CA**, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS; Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005; **352**: 2477-2486 [PMID: 15951574 DOI: 10.1056/NEJMoa042973]

3 **Martis R**, Crowther CA, Shepherd E, Alsweiler J, Downie MR, Brown J. Treatments for women with gestational diabetes mellitus: an overview of Cochrane systematic reviews. *Cochrane Database Syst Rev* 2018; **8**: CD012327 [PMID: 30103263 DOI: 10.1002/14651858.CD012327.pub2]

4 **Horvath K**, Koch K, Jeitler K, Matyas E, Bender R, Bastian H, Lange S, Siebenhofer A. Effects of treatment in women with gestational diabetes mellitus: systematic review and meta-analysis. *BMJ* 2010; **340**: c1395 [PMID: 20360215 DOI: 10.1136/bmj.c1395]

5 **Landon MB**, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, Wapner RJ, Varner MW, Rouse DJ, Thorp JM Jr, Sciscione A, Catalano P, Harper M, Saade G, Lain KY, Sorokin Y, Peaceman AM, Tolosa JE, Anderson GB; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. A multicenter, randomized trial of treatment for mild gestational diabetes. *N Engl J Med* 2009; **361**: 1339-1348 [PMID: 19797280 DOI: 10.1056/NEJMoa0902430]

6 **Cheung NW**, Moses RG. Gestational Diabetes Mellitus: Is It Time to Reconsider the Diagnostic Criteria? *Diabetes Care* 2018; **41**: 1337-1338 [PMID: 29934476 DOI: 10.2337/dci18-0013]

7 **Agarwal MM**. Gestational diabetes mellitus: An update on the current international diagnostic criteria. *World J Diabetes* 2015; **6**: 782-791 [PMID: 26131321 DOI: 10.4239/wjd.v6.i6.782]

8 **Cundy T**, Ackermann E, Ryan EA. Gestational diabetes: new criteria may triple the prevalence but effect on outcomes is unclear. *BMJ* 2014; **348**: g1567 [PMID: 24618099 DOI: 10.1136/bmj.g1567]

9 **Agarwal MM**. Consensus in Gestational Diabetes MELLITUS: Looking for the Holy Grail. *J Clin Med* 2018; **7** [PMID: 29843401 DOI: 10.3390/jcm7060123]

10 **HAPO Study Cooperative Research Group**. , Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; **358**: 1991-2002 [PMID: 18463375 DOI: 10.1056/NEJMoa0707943]

11 **International Association of Diabetes and Pregnancy Study Groups Consensus Panel**. , Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva Ad, Hod M, Kitzmiler JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt MI. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* 2010; **33**: 676-682 [PMID: 20190296 DOI: 10.2337/dc09-1848]

12 **Visser GH**, de Valk HW. Is the evidence strong enough to change the diagnostic criteria for gestational diabetes now? *Am J Obstet Gynecol* 2013; **208**: 260-264 [PMID: 23103371 DOI: 10.1016/j.ajog.2012.10.881]

13 **Moynihan R**, Doust J, Henry D. Preventing overdiagnosis: how to stop harming the healthy. *BMJ* 2012; **344**: e3502 [PMID: 22645185 DOI: 10.1136/bmj.e3502]

14 **Agarwal MM**, Dhatt GS, Othman Y. Gestational diabetes: differences between the current international diagnostic criteria and implications of switching to IADPSG. *J Diabetes Complications* 2015; **29**: 544-549 [PMID: 25837380 DOI: 10.1016/j.jdiacomp.2015.03.006]

15 **Arora GP**, Thaman RG, Prasad RB, Almgren P, Brøns C, Groop LC, Vaag AA. Prevalence and risk factors of gestational diabetes in Punjab, North India: results from a population screening program. *Eur J Endocrinol* 2015; **173**: 257-267 [PMID: 26012589 DOI: 10.1530/EJE-14-0428]

16 **Boyadzhieva MV**, Atanasova I, Zacharieva S, Tankova T, Dimitrova V. Comparative analysis of current diagnostic criteria for gestational diabetes mellitus. *Obstet Med* 2012; **5**: 71-77 [PMID: 27579139 DOI: 10.1258/om.2011.110073]

17 **Helseth R**, Salvesen O, Stafne SN, Mørkved S, Salvesen KA, Carlsen SM. Gestational diabetes mellitus among Nordic Caucasian women: prevalence and risk factors according to WHO and simplified IADPSG criteria. *Scand J Clin Lab Invest* 2014; **74**: 620-628 [PMID: 24980704 DOI: 10.3109/00365513.2014.928942]

18 **Jenum AK**, Mørkrid K, Sletner L, Vangen S, Torper JL, Nakstad B, Voldner N, Rognerud-Jensen OH, Berntsen S, Mosdøl A, Skrivarhaug T, Vårdal MH, Holme I, Yajnik CS, Birkeland KI. Impact of ethnicity on gestational diabetes identified with the WHO and the modified International Association of Diabetes and Pregnancy Study Groups criteria: a population-based cohort study. *Eur J Endocrinol* 2012; **166**: 317-324 [PMID: 22108914 DOI: 10.1530/EJE-11-0866]

19 **Koning SH**, van Zanden JJ, Hoogenberg K, Lutgers HL, Klomp AW, Korteweg FJ, van Loon AJ, Wolffenbuttel BHR, van den Berg PP. New diagnostic criteria for gestational diabetes mellitus and their impact on the number of diagnoses and pregnancy outcomes. *Diabetologia* 2018; **61**: 800-809 [PMID: 29167927 DOI: 10.1007/s00125-017-4506-x]

20 **Kun A**, Tornóczky J, Tabák AG. The prevalence and predictors of gestational diabetes mellitus in Hungary. *Horm Metab Res* 2011; **43**: 788-793 [PMID: 22009374 DOI: 10.1055/s-0031-1287795]

21 **Olagbuji BN**, Atiba AS, Olofinbiyi BA, Akintayo AA, Awoleke JO, Ade-Ojo IP, Fasubaa OB; Gestational Diabetes Study Group-Nigeria. Prevalence of and risk factors for gestational diabetes using 1999, 2013 WHO and IADPSG criteria upon implementation of a universal one-step screening and diagnostic strategy in a sub-Saharan African population. *Eur J Obstet Gynecol Reprod Biol* 2015; **189**: 27-32 [PMID: 25855324 DOI: 10.1016/j.ejogrb.2015.02.030]

22 **Pan L**, Leng J, Liu G, Zhang C, Liu H, Li M, Tan L, Tian H, Chan JC, Hu G, Yu Z, Yang X. Pregnancy outcomes of Chinese women with gestational diabetes mellitus defined by the IADPSG's but not by the 1999 WHO's criteria. *Clin Endocrinol (Oxf)* 2015; **83**: 684-693 [PMID: 25903847 DOI: 10.1111/cen.12801]

23 **Trujillo J**, Vigo A, Duncan BB, Falavigna M, Wendland EM, Campos MA, Schmidt MI. Impact of the International Association of Diabetes and Pregnancy Study Groups criteria for gestational diabetes. *Diabetes Res Clin Pract* 2015; **108**: 288-295 [PMID: 25765668 DOI: 10.1016/j.diabres.2015.02.007]

24 **Dahanayaka NJ**, Agampodi SB, Ranasinghe OR, Jayaweera PM, Wickramasinghe WA, Adhikari AN, Chathurani HK, Dissanayaka UT. Inadequacy of the risk factor based approach to detect gestational diabetes mellitus. *Ceylon Med J* 2012; **57**: 5-9 [PMID: 22453704 DOI: 10.4038/cmj.v57i1.4193]

25 **Wendland EM**, Torloni MR, Falavigna M, Trujillo J, Dode MA, Campos MA, Duncan BB, Schmidt MI. Gestational diabetes and pregnancy outcomes--a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. *BMC Pregnancy Childbirth* 2012; **12**: 23 [PMID: 22462760 DOI: 10.1186/1471-2393-12-23]

26 **Saeedi M**, Cao Y, Fadl H, Gustafson H, Simmons D. Increasing prevalence of gestational diabetes mellitus when implementing the IADPSG criteria: A systematic review and meta-analysis. *Diabetes Res Clin Pract* 2021; **172**: 108642 [PMID: 33359574 DOI: 10.1016/j.diabres.2020.108642]

27 **Ramezani Tehrani F**, Naz MSG, Yarandi RB, Behboudi-Gandevani S. The Impact of Diagnostic

Criteria for Gestational Diabetes Mellitus on Adverse Maternal Outcomes: A Systematic Review and Meta-Analysis. *J Clin Med* 2021; **10** [PMID: 33572314 DOI: 10.3390/jcm10040666]

28 **Muche AA**, Olayemi OO, Gete YK. Prevalence and determinants of gestational diabetes mellitus in Africa based on the updated international diagnostic criteria: a systematic review and meta-analysis. *Arch Public Health* 2019; **77**: 36 [PMID: 31402976 DOI: 10.1186/s13690-019-0362-0]

29 **NVOG Nederlandse Vereniging voor Gynaecologie & Obstetrie**. Diabetes Mellitus en Zwangerschap 2.0 2010. [cited 10 January 2021]. Available from: https://www.nvog.nl/wp-content/uploads/2018/02/Diabetes-mellitus-en-zwangerschap-2.0-04-06-2010.pdf

30 **Visser GH**, Eilers PH, Elferink-Stinkens PM, Merkus HM, Wit JM. New Dutch reference curves for birthweight by gestational age. *Early Hum Dev* 2009; **85**: 737-744 [PMID: 19914013 DOI: 10.1016/j.earlhumdev.2009.09.008]

31 **Deeks JJ**, Altman DG. Diagnostic tests 4: likelihood ratios. *BMJ* 2004; **329**: 168-169 [PMID: 15258077 DOI: 10.1136/bmj.329.7458.168]

32 **Eusebi P**. Diagnostic accuracy measures. *Cerebrovasc Dis* 2013; **36**: 267-272 [PMID: 24135733 DOI: 10.1159/000353863]

33 **Behboudi-Gandevani S**, Amiri M, Bidhendi Yarandi R, Ramezani Tehrani F. The impact of diagnostic criteria for gestational diabetes on its prevalence: a systematic review and meta-analysis. *Diabetol Metab Syndr* 2019; **11**: 11 [PMID: 30733833 DOI: 10.1186/s13098-019-0406-1]

34 **Kim MH**, Kwak SH, Kim SH, Hong JS, Chung HR, Choi SH, Kim MY, Jang HC. Pregnancy Outcomes of Women Additionally Diagnosed as Gestational Diabetes by the International Association of the Diabetes and Pregnancy Study Groups Criteria. *Diabetes Metab J* 2019; **43**: 766-775 [PMID: 30877713 DOI: 10.4093/dmj.2018.0192]

35 **Chi C**, Loy SL, Chan SY, Choong C, Cai S, Soh SE, Tan KH, Yap F, Gluckman PD, Godfrey KM, Shek LP, Chan JKY, Kramer MS, Chong YS. Impact of adopting the 2013 World Health Organization criteria for diagnosis of gestational diabetes in a multi-ethnic Asian cohort: a prospective study. *BMC Pregnancy Childbirth* 2018; **18**: 69 [PMID: 29562895 DOI: 10.1186/s12884-018-1707-3]

36 **Gilder ME**, Zin TW, Wai NS, Ner M, Say PS, Htoo M, Say S, Htay WW, Simpson JA, Pukrittayakamee S, Nosten F, McGready R. Gestational diabetes mellitus prevalence in Maela refugee camp on the Thai-Myanmar border: a clinical report. *Glob Health Action* 2014; **7**: 23887 [PMID: 24824580 DOI: 10.3402/gha.v7.23887]

37 **Yew TW**, Khoo CM, Thai AC, Kale AS, Yong EL, Tai ES. The Prevalence of Gestational Diabetes Mellitus Among Asian Females is Lower Using the New 2013 World Health Organization Diagnostic Criteria. *Endocr Pract* 2014; **20**: 1064-1069 [PMID: 24936548 DOI: 10.4158/EP14028.OR]

38 **Saleh L**, Shareef M, Meiracker AHVD, Visser W. The impact of implementing the WHO-2013 criteria for gestational diabetes mellitus on its prevalence and pregnancy outcomes: A comparison of the WHO-1999 and WHO-2013 diagnostic thresholds. *Eur J Obstet Gynecol Reprod Biol* 2020; **249**: 107 [PMID: 32362351 DOI: 10.1016/j.ejogrb.2020.04.019]

39 **Duran A**, Sáenz S, Torrejón MJ, Bordiú E, Del Valle L, Galindo M, Perez N, Herraiz MA, Izquierdo N, Rubio MA, Runkle I, Pérez-Ferre N, Cusihuallpa I, Jiménez S, García de la Torre N, Fernández MD, Montañez C, Familiar C, Calle-Pascual AL. Introduction of IADPSG criteria for the screening and diagnosis of gestational diabetes mellitus results in improved pregnancy outcomes at a lower cost in a large cohort of pregnant women: the St. Carlos Gestational Diabetes Study. *Diabetes Care* 2014; **37**: 2442-2450 [PMID: 24947793 DOI: 10.2337/dc14-0179]

40 **Huhn EA**, Massaro N, Streckeisen S, Manegold-Brauer G, Schoetzau A, Schulzke SM, Winzeler B, Hoesli I, Lapaire O. Fourfold increase in prevalence of gestational diabetes mellitus after adoption of the new International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria. *J Perinat Med* 2017; **45**: 359-366 [PMID: 27508951 DOI: 10.1515/jpm-2016-0099]

41 **Doust J**, Vandvik PO, Qaseem A, Mustafa RA, Horvath AR, Frances A, Al-Ansary L, Bossuyt P, Ward RL, Kopp I, Gollogly L, Schunemann H, Glasziou P; Guidelines International Network (G-I-N) Preventing Overdiagnosis Working Group. Guidance for Modifying the Definition of Diseases: A Checklist. *JAMA Intern Med* 2017; **177**: 1020-1025 [PMID: 28505266 DOI: 10.1001/jamainternmed.2017.1302]

42 **Wu ET**, Nien FJ, Kuo CH, Chen SC, Chen KY, Chuang LM, Li HY, Lee CN. Diagnosis of more gestational diabetes lead to better pregnancy outcomes: Comparing the International Association of the Diabetes and Pregnancy Study Group criteria, and the Carpenter and Coustan criteria. *J Diabetes Investig* 2016; **7**: 121-126 [PMID: 26816609 DOI: 10.1111/jdi.12378]

43 **Djaković I**, Sabolović Rudman S, Gall V, Košec A, Markuš Sandrić M, Košec V. Do Changing Diagnostic Criteria for Gestational Diabetes Influence Pregnancy Outcome? *Acta Clin Croat* 2016; **55**: 422-427 [PMID: 29045107 DOI: 10.20471/acc.2016.55.03.11]

44 **Hung TH**, Hsieh TT. The effects of implementing the International Association of Diabetes and Pregnancy Study Groups criteria for diagnosing gestational diabetes on maternal and neonatal outcomes. *PLoS One* 2015; **10**: e0122261 [PMID: 25756838 DOI: 10.1371/journal.pone.0122261]

45 **Amsterdam UMC Leading the Change the Netherlands**. TANGO-DM Study. [cited 10 January 2021]. Available from: https://zorgevaluatienederland.nl/tango-dm/study-information

46 **Crowther CA**, McCowan LME, Rowan JA, Edlin R, McKinlay CJD; GEMS Study Group. Lower *vs* higher diagnostic criteria for the detection of gestational diabetes for reducing maternal and perinatal morbidity: study protocol for the GEMS randomised trial. *BMC Pregnancy Childbirth* 2020; **20**: 547 [PMID: 32948138 DOI: 10.1186/s12884-020-03252-9]

47 **Marchetti D**, Carrozzino D, Fraticelli F, Fulcheri M, Vitacolonna E. Quality of Life in Women with

Gestational Diabetes Mellitus: A Systematic Review. *J Diabetes Res* 2017; **2017**: 7058082 [PMID: 28326332 DOI: 10.1155/2017/7058082]

48 **Carter SM**, Rogers W, Heath I, Degeling C, Doust J, Barratt A. The challenge of overdiagnosis begins with its definition. *BMJ* 2015; **350**: h869 [PMID: 25740625 DOI: 10.1136/bmj.h869]

49 **Parsons J**, Sparrow K, Ismail K, Hunt K, Rogers H, Forbes A. Experiences of gestational diabetes and gestational diabetes care: a focus group and interview study. *BMC Pregnancy Childbirth* 2018; **18**: 25 [PMID: 29325518 DOI: 10.1186/s12884-018-1657-9]

50 **Kalra B**, Gupta Y, Baruah MP. Renaming gestational diabetes mellitus: A psychosocial argument. *Indian J Endocrinol Metab* 2013; **17**: S593-S595 [PMID: 24910817 DOI: 10.4103/2230-8210.123539]

51 **Lawrence JM**. Women with diabetes in pregnancy: different perceptions and expectations. *Best Pract Res Clin Obstet Gynaecol* 2011; **25**: 15-24 [PMID: 21115403 DOI: 10.1016/j.bpobgyn.2010.10.003]

52 **Ng E**, Neff M, Sztal-Mazer S. Insights uncovered from experiencing a rise in the incidence of gestational diabetes at a Melbourne hospital. *Diabetologia* 2018; **61**: 1881-1883 [PMID: 29704118 DOI: 10.1007/s00125-018-4631-1]

53 **Lenoir-Wijnkoop I**, van der Beek EM, Garssen J, Nuijten MJ, Uauy RD. Health economic modeling to assess short-term costs of maternal overweight, gestational diabetes, and related macrosomia - a pilot evaluation. *Front Pharmacol* 2015; **6**: 103 [PMID: 26042038 DOI: 10.3389/fphar.2015.00103]

54 **Farrar D**, Simmonds M, Griffin S, Duarte A, Lawlor DA, Sculpher M, Fairley L, Golder S, Tuffnell D, Bland M, Dunne F, Whitelaw D, Wright J, Sheldon TA. The identification and treatment of women with hyperglycaemia in pregnancy: an analysis of individual participant data, systematic reviews, meta-analyses and an economic evaluation. *Health Technol Assess* 2016; **20**: 1-348 [PMID: 27917777 DOI: 10.3310/hta20860]

55 **Cade TJ**, Polyakov A, Brennecke SP. Implications of the introduction of new criteria for the diagnosis of gestational diabetes: a health outcome and cost of care analysis. *BMJ Open* 2019; **9**: e023293 [PMID: 30612109 DOI: 10.1136/bmjopen-2018-023293]

56 **Jacklin PB**, Maresh MJ, Patterson CC, Stanley KP, Dornhorst A, Burman-Roy S, Bilous RW. A cost-effectiveness comparison of the NICE 2015 and WHO 2013 diagnostic criteria for women with gestational diabetes with and without risk factors. *BMJ Open* 2017; **7**: e016621 [PMID: 28801424 DOI: 10.1136/bmjopen-2017-016621]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 883-892

DOI: 10.4239/wjd.v12.i6.883

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Observational Study

Control of modifiable risk factors and major adverse cardiovascular events in people with peripheral artery disease and diabetes

Jonathan Golledge, Aaron Drovandi, Sophie Rowbotham, Ramesh Velu, Frank Quigley, Jason Jenkins

ORCID number: Jonathan Golledge 0000-0002-5779-8848; Aaron Drovandi 0000-0002-8682-1218; Sophie Rowbotham 0000-0002-4798-249X; Ramesh Velu 0000-0002-0051-4912; Frank Quigley 0000-0002-2877-8194; Jason Jenkins 0000-0001-5535-8526.

Author contributions: Golledge J was the guarantor; Golledge J, Rowbotham S, Quigley F, Velu R, and Jenkins J designed the study; Golledge J, Rowbotham S, Quigley F, Velu R, and Jenkins J participated in data collection; Golledge J and Drovandi A analysed and interpreted the data, and drafted the initial manuscript; All authors reviewed and approved the final manuscript version.

Supported by The National Health and Medical Research Council, No. 1063476 and No. 1022752; James Cook University and Queensland Government supported this work. JG holds a Practitioner Fellowships from the National Health and Medical Research Council, No. 1117061.

Institutional review board statement: The study was performed in accordance with the Helsinki declaration and ethical approval was granted from the James Cook University and

Jonathan Golledge, Aaron Drovandi, Sophie Rowbotham, Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville 4811, Queensland, Australia

Jonathan Golledge, Ramesh Velu, Department of Vascular and Endovascular Surgery, Townsville University Hospital, Townsville 4811, Queensland, Australia

Frank Quigley, Department of Vascular Surgery, Mater Hospital, Townsville 4811, Queensland, Australia

Jason Jenkins, Department of Vascular Surgery, The Royal Brisbane and Women's Hospital, Brisbane 4000, Queensland, Australia

Corresponding author: Jonathan Golledge, MChir, Director, Professor, Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, 100 Angus Smith Drive, Townsville 4811, Queensland, Australia.
jonathan.golledge@jcu.edu.au

Abstract

BACKGROUND

People with diabetes and peripheral artery disease (PAD) have a high risk of major adverse cardiovascular events (MACE). Prior research suggests that medical therapies aimed to control modifiable risk factors are poorly implemented in patients with PAD.

AIM

To examine the association between the control of modifiable risk factors, estimated by the novel PAD-medical score, and the incidence of MACE in people with PAD and diabetes.

METHODS

Participants were recruited from out-patient clinics if they had a diagnosis of both PAD and diabetes. Control of reversible risk factors was assessed by a new composite measure, the PAD-medical score. This score takes into account the control of low-density lipoprotein cholesterol, blood pressure, blood glucose, smoking and prescription of an anti-platelet. Participants were followed to record incidence of myocardial infarction, stroke and cardiovascular death (MACE). The association of PAD-medical score with MACE was assessed using Cox propor-

Townsville Hospital Health Service human research ethics committees (HREC/13/QTHS/125 and HREC/14/QTHS/203).

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

Data sharing statement: The main data from this study was presented in the manuscript. Individual participant data cannot be provided due to regulations inherent in a legal agreement required as a result of the governance approval covering the data and ethics of the project. Data are available from the corresponding author upon reasonable request.

STROBE statement: The authors have read the STROBE Statement, and the manuscript was prepared and revised according to the STROBE Statement.

Manuscript source: Unsolicited Manuscript

Specialty type: Peripheral Vascular Disease

Country/Territory of origin: Australia

Peer-review report's scientific quality classification

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

tional hazard analyses adjusting for age, sex and prior history of ischemic heart disease and stroke.

RESULTS

Between 2002 and 2020, a total of 424 participants with carotid artery disease (n = 63), aortic or peripheral aneurysm (n = 121) or lower limb ischemia (n = 240) were prospectively recruited, and followed for a median duration (inter-quartile range) of 2.0 (0.2–4.4) years. Only 33 (7.8%) participants had the optimal PAD-medical score of five, with 318 (75%) scoring at least three out of five. There were 89 (21.0%) participants that had at least one MACE during the follow-up period. A one-unit higher PAD-medical score was associated with lower risk of MACE (HR = 0.79, 95%CI: 0.63-0.98) after adjusting for other risk factors.

CONCLUSION

The PAD-medical score provides a simple way to assess the control of modifiable risk factors targeted by medical management aimed to reduce the incidence of MACE.

Key Words: Peripheral artery disease; Diabetes; Major cardiovascular events; Medical management; Prospective study; Clinical practice

Core Tip: The control of modifiable risk factors for major adverse cardiovascular events (MACE) is frequently poorly achieved in patients with peripheral artery disease (PAD). The PAD-medical score is an easy way to assess the control of modifiable risk factors. In the current study only 33 (7.8%) of the included participants had optimal control of risk factors evidenced by a maximum PAD-medical score. Adjusted analyses found that a one-unit higher PAD-medical score was associated with a significantly lower risk of MACE (HR = 0.79, 95%CI: 0.63-0.98).

Citation: Golledge J, Drovandi A, Rowbotham S, Velu R, Quigley F, Jenkins J. Control of modifiable risk factors and major adverse cardiovascular events in people with peripheral artery disease and diabetes. *World J Diabetes* 2021; 12(6): 883-892
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/883.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.883

INTRODUCTION

Diseases of the aorta and its branches (peripheral artery disease; PAD) are a collection of chronic occlusive and aneurysmal diseases, such as carotid artery disease, abdominal aortic aneurysm and lower limb ischemia[1-4]. Depending on study entry criteria and testing, between 10% and 60% of people with PAD have been reported to have diabetes[5]. Poorly controlled diabetes, as estimated by high hemoglobin A1c (HbA1c) concentrations, has been associated with an increased risk of major adverse cardiovascular events (MACE; myocardial infarction, stroke or cardiovascular death) in people with PAD[6,7], with approximately 20% having events during short-term follow-up[8-13].

Randomised controlled trials have demonstrated that medical therapies that control key cardiovascular risk factors, such as reducing low density lipoprotein-cholesterol (LDL-c) concentrations[11,14], blood pressure[15] and blood glucose[16], and reducing the risk of thrombosis[17], are effective at substantially reducing the risk of MACE. In clinical practice, however these therapies are poorly implemented[5,8,18-20]. Prior studies have suggested that poor implementation of medical therapies are associated with a higher incidence of MACE, but are limited through largely focusing on assessing the control of individual risk factors, such as blood pressure or LDL-c alone [8,18,19]. A holistic assessment of implementation of medical therapies amongst people with PAD and diabetes would consider control of all key modifiable risk factors.

Received: January 19, 2021
Peer-review started: January 19, 2021
First decision: February 25, 2021
Revised: March 8, 2021
Accepted: May 20, 2021
Article in press: May 20, 2021
Published online: June 15, 2021

P-Reviewer: Nakajima K, Vela D
S-Editor: Ma YJ
L-Editor: A
P-Editor: Yuan YY

The first aim of this study was to examine the implementation of all the key medical therapies in accordance with current clinical guidelines in a group of people that had both PAD and diabetes. This included achieving optimal control of serum LDL-c, systolic blood pressure, HbA1c, smoking abstinence and prescription of an anti-platelet medication[21-23]. This was measured through the introduction of a new algorithm, PAD-medical, designed to quantify the relative control of the key modifiable risk factors targeted by optimal medical management. Secondly, this study aimed to examine the association of implementation of these combined medical therapies, estimated by the relative control of modifiable risk factors using PAD-medical score, with the incidence of MACE, adjusted for other risk factors.

MATERIALS AND METHODS

Study design and participants

This investigation was designed as part of an ongoing prospective cohort study that commenced in 2002, and aimed to identify risk factors associated with PAD outcomes [24,25]. The current study included adult participants (≥ 18 years old) with a prior history of a diagnosis of both diabetes and PAD, presenting to the outpatient vascular services at The Townsville University Hospital, the Mater Hospital Townsville and The Royal Brisbane and Women's Hospital in Queensland, Australia. People presenting with carotid artery disease, an abdominal or peripheral aneurysm or lower limb PAD diagnosed by a vascular specialist were eligible as long as they had a prior diagnosis of diabetes based on documented medical records from previous visits[26,27]. Participants presenting without diagnoses of both diseases were excluded.

Lower limb PAD was defined to include symptoms of leg or foot pain or tissue loss and absence of lower limb pulses, ankle-brachial index ≤ 0.9 or imaging evidence of a lower limb artery stenosis of ≥ 50% or occlusion[24,25]. Abdominal aortic aneurysm (AAA) was diagnosed if the orthogonal maximum outer to outer infra-renal aortic wall diameter was ≥ 30 mm measured from ultrasound or computed tomographic angiography[24,25]. Peripheral aneurysms were defined to include common or internal iliac artery diameters ≥ 15 and ≥ 8mm respectively, or femoral or popliteal artery diameters of ≥ 15 mm and ≥ 9 mm respectively, as previously described[28]. A significant carotid artery stenosis was defined as ≥ 50% using Australian Society for Ultrasound in Medicine criteria[24,25]. Written informed consent was obtained from all participants upon entry into the study. The study was performed in accordance with the Helsinki declaration and ethical approval was granted from the James Cook University and Townsville Hospital Health Service human research ethics committees (HREC/13/QTHS/125 and HREC/14/QTHS/203).

Risk factors and assessment of medical management

The implementation of medical management was assessed using a composite measure, the PAD-medical score, which was developed for this study in order to assess the control of the key modifiable risk factors targeted to reduce the risk of MACE[5,8,18,29-31]. This scoring tool was developed to address the lack of an existing tool which applies to all age groups, and includes the relevant medical risk factors for people with concomitant PAD and diabetes (*e.g.*, HbA1c). The PAD-medical has a possible score of between 0 and 5, with zero indicating worst implementation of medical management and five indicating best implementation of medical manage-ment.

PAD-medical was calculated based on the control of key risk factors, smoking history and anti-platelet prescription measured at study entry. PAD-medical used risk factor targets for preventing MACE in patients with PAD indicated by current clinical guidelines[21-23], with each target achieved scoring one point. Values for some risk factors indicating partial control were scored either 0.25 or 0.5 points. The PAD-medical score was thus calculated as follows: Serum LDL-C: ≥ 3.0 mmol/L = 0, 2.5-2.9 mmol/L = 0.25; 1.8-2.4 mmol/L = 0.5; < 1.8 mmol/L = 1; Systolic blood pressure: > 160 mmHg = 0; 140-160 mmHg = 0.5; < 140 mmHg = 1; HbA1c: > 9% = 0; 7%-9% = 0.5; < 7% = 1; Smoking history: not smoked within the last month = 1; smoked within the last month = 0; Prescribed an anti-platelet medication: confirmed receiving = 1; not receiving = 0.

Definition and assessment of outcomes

At entry, participants underwent fasting blood tests, resting blood pressure was measured using an Omron Intellisense (HEM-907) monitor and smoking history and prescribed medications were recorded[8]. All prescribed medications including

antiplatelet drugs, statins and diabetes medications were recorded. Serum LDL-C, HbA1c and C-reactive protein were measured as previously described[27,32]. Ischemic heart disease (IHD) was defined as a history of myocardial infarction, angina or previous treatment for IHD[33].

Participants were offered follow up annually as part of standard care, and outpatient follow up was performed according to local clinical practice. Outcome data were recorded during clinical reviews on prospectively defined case report forms. Hospital charts and electronic records were also reviewed by a vascular specialist. Outcome data were also obtained from linked hospital admission records as previously described[24,25,34,35]. Linked data were obtained from the Queensland Hospital Admitted Patient Data Collection (QHAPDC) which is regularly audited to minimize inaccuracies[36]. The primary outcome was MACE, defined as the first occurrence of a major cardiovascular event including myocardial infarction, stroke or cardiovascular death.

Sample size

It was aimed to have adequate power to test the hypothesis that the PAD-medical score was associated with the risk of MACE. Previous studies suggest that approximately 30% of people with PAD have a MACE during short term follow-up[8,9,34]. Monte-Carlo simulations suggest that a multivariable regression model is powered sufficiently when 10 outcome events per degree of freedom of the predictor variables are observed[37]. Assuming an incidence of MACE of 20% to 30%, and planning to adjust for PAD-medical score (a composite of five risk factors), age, sex, IHD and stroke in the regression models, it was estimated that a sample size of over 400 participants would have adequate power to test the main hypothesis.

Statistical analysis

Data were analysed using the SPSS v25 (IBM, Armonk, NY, United States) software package. Continuous data that were not normally distributed, as confirmed using the Shapiro Wilk test, were presented as median and inter-quartile range (IQR). Between-group comparisons were conducted using the Mann-Whitney *U* and Kruskal-Wallis tests. Categorical variables were compared using Pearson's χ^2 test. Cox proportional hazard analyses assessed the association of PAD-medical score (one unit increase) with MACE adjusted for age, sex, smoking, IHD and prior stroke. Results were presented as hazard ratios (HR) and 95% confidence intervals (CI). *P* values of < 0.05 were accepted to be significant for all of these analyses.

RESULTS

Participants and implementation of medical management

Between February 2002 and August 2020, 424 participants with comorbid diabetes and PAD presenting with carotid artery disease ($n = 63$), aortic or peripheral aneurysm ($n = 121$) or lower limb ischemia ($n = 240$) were recruited. Only 33 (7.8%) of the participants had the optimal PAD-medical score of 5, with 173 (40.8%) scoring ≥ 4, and 318 (75.0%) scoring ≥ 3. Sex, age and history of IHD varied significantly between participants with different PAD-medical scores (Table 1). As expected, participants with the higher PAD-medical scores had significantly lower HbA1c, LDL-c and systolic blood pressure, reduced frequency of current smoking, and more frequent prescription of anti-platelet and statin medications (Table 2).

Association of PAD-medical score with MACE

Participants were followed for a median of 2.0 (0.2-4.4) years. During this time, 89 (21.0%) participants had at least one MACE. Overall, 43 participants had a myocardial infarction, 20 had a stroke and 51 a cardiovascular-related death, with 27 participants having multiple events. In unadjusted analyses, there was no relationship between MACE and PAD-medical score per unit increase (HR = 0.90, 95%CI: 0.73-1.11). In analyses adjusted for age, sex, IHD and stroke (Table 3), higher PAD-medical scores were associated with a significantly lower risk of MACE per unit increase (HR = 0.79, 95%CI: 0.63-0.98). Of the components of the PAD-medical score only smoking abstinence was significantly associated with a lower risk of MACE per unit increase (HR = 0.61, 95%CI: 0.38-0.97).

Table 1 Characteristics of include participants (*n* = 424)

Characteristic	Value
Age (median years, IQR)	69 (63-76)
Gender (male, %)	311 (73.3)
Aboriginal or Torres strait islander, *n* (%)	19 (4.5)
Presenting problem, *n* (%)	
Carotid artery disease	63 (14.9)
Aortic or peripheral aneurysm	121 (28.5)
Lower limb ischemia	240 (56.6)
Smoking status, *n* (%)	
Current	101 (23.8)
Former	211 (49.8)
Never	111 (26.2)
Missing	1 (0.2)
Medications, *n* (%)	
Aspirin	278 (65.6)
Other anti-platelet	84 (19.8)
Statins	324 (76.4)
Metformin	251 (59.2)
Other oral hypoglycemics	154 (36.3)
Insulin	98 (23.1)
Co-morbidities, *n* (%)	
Ischemic heart disease (IHD)	201 (47.4)
Stroke	47 (11.1)
Vitals (median, IQR)	
Glycated hemoglobin (HbA1c) (%)	6.9 (6.2-7.8)
Low-density lipoprotein (LDL)-c (mmol/L)	2 (1.5-2.6)
Systolic blood pressure (mmHg)	139 (125-151)
C-reactive protein (mg/L)[1]	2.7 (1.0-6.0)

[1]C-reactive protein data is missing from 37 participants.

DISCUSSION

This study illustrates the considerable rate of clinically-important events in people that have PAD and diabetes, with 21% having at least one MACE during a median follow-up of 2 years. The main finding of the study was that most people with diabetes and PAD do not have optimal control of modifiable risk factors for MACE. Participants with better implementation of medical management, as identified by higher PAD-medical scores, had a lower of risk of MACE after adjusting for other risk factors. However, in sub-analyses, only smoking abstinence was found to be associated with a significantly reduced risk of MACE. The findings emphasize the need for methods to better implement medical management in people with PAD, particularly smoking cessation. The study also introduces a simple to use way to measure the overall success of control of modifiable risk factors using PAD-medical.

Previous studies show that people presenting with PAD have a higher risk of MACE than those frequently considered to be at the highest risk, such as those who have had a myocardial infarction or stroke[11]. Thus there is a need to develop strategies that improve the implementation of evidence-based medical therapies that are effective at reducing the incidence of MACE in people with PAD. Strategies to

Table 2 Association of peripheral artery disease-medical score with baseline characteristics

Risk factors	PAD-medical score				*P* value
	< 3.0 (*n* = 106)	3-3.9 (*n* = 145)	4.0-4.9 (*n* = 140)	5.0 (*n* = 33)	
Age	65 (57-73)	70 (64-77)	69 (64-77)	70 (66-76)	0.002[b]
Male sex	67 (63.2)	110 (75.9)	110 (78.6)	24 (72.7)	0.046[a]
Aboriginal or Torres Strait Islander	3 (2.8)	9 (6.2)	4 (2.9)	3 (9.1)	0.242
Presenting problem					0.043[a]
Carotid artery disease	12 (11.3)	19 (13.1)	27 (19.3)	5 (15.2)	
Aortic or peripheral aneurysm	22 (20.8)	45 (31.0)	42 (30.0)	12 (36.4)	
Lower limb ischemia	72 (67.9)	81 (55.9)	71 (50.7)	16 (48.5)	
Smoking status					< 0.001[c]
Current	55 (51.9)	41 (28.3)	5 (3.6)	0	
Former	28 (26.4)	62 (42.8)	100 (71.4)	21 (63.6)	
Never	22 (20.8)	42 (29.0)	35 (25.0)	12 (36.4)	
IHD	38 (35.8)	64 (44.1)	75 (53.6)	24 (72.7)	0.001[b]
Stroke	14 (13.2)	12 (8.3)	17 (12.1)	4 (12.1)	0.606
Medications					
Aspirin	34 (32.1)	96 (66.2)	118 (84.3)	30 (90.9)	< 0.001[c]
Other anti-platelet	11 (10.4)	25 (17.2)	41 (29.3)	7 (21.2)	0.002[b]
Statins	58 (54.7)	115 (79.3)	121 (86.4)	30 (90.9)	< 0.001[c]
Metformin	60 (56.6)	87 (60.0)	83 (59.3)	21 (63.6)	0.896
Other oral hypoglycemics	39 (36.8)	58 (40.0)	45 (32.1)	12 (36.4)	0.591
Insulin	26 (24.5)	35 (24.1)	33 (23.6)	4 (12.1)	0.482
HbA1c (%)	7.4 (6.8-8.7)	7.0 (6.5-7.7)	6.6 (6.0-7.6)	6.1 (5.9-6.4)	< 0.001[c]
HDL-c (mmol/L)	1.1 (0.9-1.3)	1.1(0.9-1.3)	1.1(0.9-1.2)	0.9(0.8-1.2)	0.133
LDL-c (mmol/L)	2.60 (2.20-3.40)	2.10 (1.70-2.70)	1.60 (1.30-2.00)	1.34 (1.20-1.60)	< 0.001[c]
SBP (mmHg)	150 (139-163)	139 (125-151)	135 (122-150)	126 (120-131)	< 0.001[c]
C-reactive protein (mg/L)	3.0 (1.9-7.5)[1]	2.0 (1.0-5.0)[2]	3.0 (1.0-5.6)[3]	2.1 (0.9-5.0)[4]	0.082
Major cardiovascular events	23 (21.7)	27 (18.6)	32 (22.9)	7 (21.2)	0.845

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
Data are presented as number (percentage) or median (interquartile range). C-reactive protein data is missing from 9[1], 15[2], 8[3] and 5[4] participants. PAD: Peripheral artery disease; IHD: Ischemic heart disease; HbA1c: Hemoglobin A1c; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SBP: Systolic blood pressure.

promote smoking abstinence in particular may be of highest priority given its association with a reduced risk of MACE in this study. It is however currently unclear as to what intervention strategies would be effective in achieving better implementation of these measures amongst people with PAD. Possible strategies include physician and patient education programs and technology-enabled reminder systems for medications adherence [19,38]. In order to test such interventions and their contribution to achieving optimal medical management, there is a need for a simple scoring system to assess overall risk factor control which PAD-medical can provide.

Several models have been designed for predicting the incidence of MACE, such as the Framingham risk score, however these were mainly developed for use in primary rather than secondary prevention. The SMART-REACH model was developed for use in assessment of risk amongst people with established cardiovascular disease and has

Table 3 Association of peripheral artery disease-medical score and components with risk of major adverse cardiovascular events

Risk factor measure	HR	95%CI	*P* value
PAD-medical score	0.79	0.63 to 0.98	0.030[a]
LDL-C score	0.80	0.46 to 1.39	0.436
HbA1c score	0.94	0.48 to 1.83	0.853
Blood pressure score	0.59	0.30 to 1.16	0.125
Smoking abstinence	0.61	0.38 to 0.97	0.036[a]
Any anti-platelet	0.81	0.49 to 1.34	0.411

[a]$P < 0.05$. Adjusted for age, sex, ischemic heart disease and stroke. PAD: Peripheral artery disease; LDL: Low-density lipoprotein; HbA1c: Hemoglobin A1c.

been used to model risk of MACE amongst people with PAD[39]. The SMART-REACH model, however, has a number of weaknesses in the assessment of the implementation of optimal medical management. These include the incorporation of risk factors not impacted by medical management, like age and sex, the lack of inclusion of key medical targets within the score calculation, such as HbA1c, and the ineligibility of some participants for the score, such as people older than 80 years. The PAD-medical score developed in this study aims to address these issues by strictly focusing on the medical management and risk factors targeted by medical management. As demonstrated in this study the PAD-medical score provides a convenient way to assess how well medical management is implemented, how modifiable risk factors are controlled and also the risk of MACE. The PAD-medical score may therefore be useful in the assessment of people with PAD.

The current study has a number of limitations that should be noted. Firstly, the sample size included was relatively small and recruited from one state in Australia. Secondly, a heterogeneous group of different PAD presentations was included. Thirdly, there was short median duration of participant follow-up. Fourthly, sub-analyses suggested a single risk factor (smoking abstinence) was associated with a lower risk of MACE. Finally, while the scoring system for PAD-medical was developed after considering current clinical guidelines targets[21-23], the cut-off values for the intermediate categories were set arbitrary. As a result of this and the small sample size, the findings of this study need to be validated in a more diverse population recruited from other localities, with a longer duration of follow-up.

CONCLUSION

This study illustrates the high incidence of clinically important events in people with PAD and diabetes. A simple-to-calculate score called PAD-medical is presented, which can be used to assess how well medical management therapy achieves control of modifiable risk factors. The PAD-medical score was predictive of the incidence of MACE during short-term follow-up.

ARTICLE HIGHLIGHTS

Research background

Peripheral artery disease is collection of chronic occlusive and aneurysmal diseases associated with a high incidence of major adverse cardiovascular events (MACE).

Research motivation

In this study, the control of modifiable risk factors for MACE was assessed through the development and testing of a new score called peripheral artery disease (PAD)-medical.

Research objectives

The aim of this study was to assess how the PAD-medical score, which assessed the control of modifiable risk factors was associated with the risk of MACE in people with

a diagnosis of both peripheral artery disease and diabetes.

Research methods

Patients with previously diagnosed peripheral artery disease and diabetes were recruited from three hospitals in Queensland Australia. PAD-medical score was calculated as a result from zero (worst management) to five (best management) based on the control of modifiable risk factors and implementation of medical management. Cox proportional hazard analyses assessed the association of PAD-medical score (one unit increase) with MACE adjusted for age, sex, smoking, IHD and prior stroke.

Research results

Of 424 participants recruited less than 10% had optimal control of modifiable risk factors evidenced by a top PAD-medical score. A one-unit increase in PAD-medical was associated with a significantly lower risk of MACE after adjusting for other risk factors (HR = 0.79, 95%CI: 0.63-0.98). Of the five different components of PAD-medical, only smoking abstinence was independently associated with a reduced risk of MACE (HR = 0.61, 95%CI: 0.38-0.97).

Research conclusions

The PAD-medical score represents an easy to use tool for the quantification of the control of modifiable risk factors for MACE in patients with peripheral artery disease and diabetes.

Research perspectives

Further research into this field requires a larger participant cohort from a more diverse population to investigate the wider applicability of the PAD-medical score.

REFERENCES

1 **Fowkes FG**, Aboyans V, Fowkes FJ, McDermott MM, Sampson UK, Criqui MH. Peripheral artery disease: epidemiology and global perspectives. *Nat Rev Cardiol* 2017; **14**: 156-170 [PMID: 27853158 DOI: 10.1038/nrcardio.2016.179]

2 **Fowkes FG**, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, Norman PE, Sampson UK, Williams LJ, Mensah GA, Criqui MH. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet* 2013; **382**: 1329-1340 [PMID: 23915883 DOI: 10.1016/S0140-6736(13)61249-0]

3 **Sampson UK**, Norman PE, Fowkes FG, Aboyans V, Yanna Song, Harrell FE Jr, Forouzanfar MH, Naghavi M, Denenberg JO, McDermott MM, Criqui MH, Mensah GA, Ezzati M, Murray C. Global and regional burden of aortic dissection and aneurysms: mortality trends in 21 world regions, 1990 to 2010. *Glob Heart* 2014; **9**: 171-180.e10 [PMID: 25432126 DOI: 10.1016/j.gheart.2013.12.010]

4 **Golledge J**. Lower-limb arterial disease. *Lancet* 1997; **350**: 1459-1465 [PMID: 9371181 DOI: 10.1016/S0140-6736(97)07421-7]

5 **Nastasi DR**, Smith JR, Moxon JV, Trollope A, Golledge J. Prescription of Pharmacotherapy and the Incidence of Stroke in Patients With Symptoms of Peripheral Artery Disease. *Stroke* 2018; **49**: 2953-2960 [PMID: 30571405 DOI: 10.1161/STROKEAHA.118.022922]

6 **Singh N**, Zeng C, Lewinger JP, Wolfson AM, Shavelle D, Weaver F, Garg PK. Preoperative hemoglobin A1c levels and increased risk of adverse limb events in diabetic patients undergoing infrainguinal lower extremity bypass surgery in the Vascular Quality Initiative. *J Vasc Surg* 2019; **70**: 1225-1234.e1 [PMID: 30852042 DOI: 10.1016/j.jvs.2018.12.041]

7 **Low Wang CC**, Blomster JI, Heizer G, Berger JS, Baumgartner I, Fowkes FGR, Held P, Katona BG, Norgren L, Jones WS, Lopes RD, Olin JW, Rockhold FW, Mahaffey KW, Patel MR, Hiatt WR; EUCLID Trial Executive Committee and Investigators. Cardiovascular and Limb Outcomes in Patients With Diabetes and Peripheral Artery Disease: The EUCLID Trial. *J Am Coll Cardiol* 2018; **72**: 3274-3284 [PMID: 30573030 DOI: 10.1016/j.jacc.2018.09.078]

8 **Thomas Manapurathe D**, Moxon JV, Krishna SM, Rowbotham S, Quigley F, Jenkins J, Bourke M, Bourke B, Jones RE, Golledge J. Cohort Study Examining the Association Between Blood Pressure and Cardiovascular Events in Patients With Peripheral Artery Disease. *J Am Heart Assoc* 2019; **8**: e010748 [PMID: 30845872 DOI: 10.1161/JAHA.118.010748]

9 **Morris DR**, Skalina TA, Singh TP, Moxon JV, Golledge J. Association of Computed Tomographic Leg Muscle Characteristics With Lower Limb and Cardiovascular Events in Patients With Peripheral Artery Disease. *J Am Heart Assoc* 2018; **7**: e009943 [PMID: 30371256 DOI: 10.1161/JAHA.118.009943]

10 **Bonaca MP**, Bhatt DL, Storey RF, Steg PG, Cohen M, Kuder J, Goodrich E, Nicolau JC, Parkhomenko A, López-Sendón J, Dellborg M, Dalby A, Špinar J, Aylward P, Corbalán R, Abola MTB, Jensen EC, Held P, Braunwald E, Sabatine MS. Ticagrelor for Prevention of Ischemic Events

After Myocardial Infarction in Patients With Peripheral Artery Disease. *J Am Coll Cardiol* 2016; **67**: 2719-2728 [PMID: 27046162 DOI: 10.1016/j.jacc.2016.03.524]

11 **Bonaca MP**, Nault P, Giugliano RP, Keech AC, Pineda AL, Kanevsky E, Kuder J, Murphy SA, Jukema JW, Lewis BS, Tokgozoglu L, Somaratne R, Sever PS, Pedersen TR, Sabatine MS. Low-Density Lipoprotein Cholesterol Lowering With Evolocumab and Outcomes in Patients With Peripheral Artery Disease: Insights From the FOURIER Trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk). *Circulation* 2018; **137**: 338-350 [PMID: 29133605 DOI: 10.1161/CIRCULATIONAHA.117.032235]

12 **Anand SS**, Caron F, Eikelboom JW, Bosch J, Dyal L, Aboyans V, Abola MT, Branch KRH, Keltai K, Bhatt DL, Verhamme P, Fox KAA, Cook-Bruns N, Lanius V, Connolly SJ, Yusuf S. Major Adverse Limb Events and Mortality in Patients With Peripheral Artery Disease: The COMPASS Trial. *J Am Coll Cardiol* 2018; **71**: 2306-2315 [PMID: 29540326 DOI: 10.1016/j.jacc.2018.03.008]

13 **Golledge J**, Moxon JV, Rowbotham S, Pinchbeck J, Yip L, Velu R, Quigley F, Jenkins J, Morris DR. Risk of major amputation in patients with intermittent claudication undergoing early revascularization. *Br J Surg* 2018; **105**: 699-708 [PMID: 29566427 DOI: 10.1002/bjs.10765]

14 **Heart Protection Study Collaborative Group**. Randomized trial of the effects of cholesterol-lowering with simvastatin on peripheral vascular and other major vascular outcomes in 20,536 people with peripheral arterial disease and other high-risk conditions. *J Vasc Surg* 2007; **45**: 645-654; discussion 653 [PMID: 17398372 DOI: 10.1016/j.jvs.2006.12.054]

15 **Ostergren J**, Sleight P, Dagenais G, Danisa K, Bosch J, Qilong Y, Yusuf S; HOPE study investigators. Impact of ramipril in patients with evidence of clinical or subclinical peripheral arterial disease. *Eur Heart J* 2004; **25**: 17-24 [PMID: 14683738 DOI: 10.1016/j.ehj.2003.10.033]

16 **Goldman MP**, Clark CJ, Craven TE, Davis RP, Williams TK, Velazquez-Ramirez G, Hurie JB, Edwards MS. Effect of Intensive Glycemic Control on Risk of Lower Extremity Amputation. *J Am Coll Surg* 2018; **227**: 596-604 [PMID: 30336205 DOI: 10.1016/j.jamcollsurg.2018.09.021]

17 **Wong PF**, Chong LY, Mikhailidis DP, Robless P, Stansby G. Antiplatelet agents for intermittent claudication. *Cochrane Database Syst Rev* 2011; CD001272 [PMID: 22071801 DOI: 10.1002/14651858.CD001272.pub2]

18 **Nastasi DR**, Moxon JV, Norman R, Trollope AF, Rowbotham S, Quigley F, Jenkins J, Golledge J. The cost-effectiveness of intensive low-density lipoprotein cholesterol lowering in people with peripheral artery disease. *J Vasc Surg* 2021; **73**: 1396-1403.e3 [PMID: 32891803 DOI: 10.1016/j.jvs.2020.08.129]

19 **Burnier M**, Egan BM. Adherence in Hypertension. *Circ Res* 2019; **124**: 1124-1140 [PMID: 30920917 DOI: 10.1161/CIRCRESAHA.118.313220]

20 **World Health Organization**. Adherence to long-term therapies: evidence for action. 2003. Available from: https://www.who.int/chp/knowledge/publications/adherence_report/en/

21 **Aboyans V**, Ricco JB, Bartelink MEL, Björck M, Brodmann M, Cohnert T, Collet JP, Czerny M, De Carlo M, Debus S, Espinola-Klein C, Kahan T, Kownator S, Mazzolai L, Naylor AR, Roffi M, Röther J, Sprynger M, Tendera M, Tepe G, Venermo M, Vlachopoulos C, Desormais I, Document Reviewers, Widimsky P, Kolh P, Agewall S, Bueno H, Coca A, De Borst GJ, Delgado V, Dick F, Erol C, Ferrini M, Kakkos S, Katus HA, Knuuti J, Lindholt J, Mattle H, Pieniazek P, Piepoli MF, Scheinert D, Sievert H, Simpson I, Sulzenko J, Tamargo J, Tokgozoglu L, Torbicki A, Tsakountakis N, Tuñón J, Vega de Ceniga M, Windecker S, Zamorano JL. Editor's Choice - 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg* 2018; **55**: 305-368 [PMID: 28851596 DOI: 10.1016/j.ejvs.2017.07.018]

22 **Gerhard-Herman MD**, Gornik HL, Barrett C, Barshes NR, Corriere MA, Drachman DE, Fleisher LA, Fowkes FGR, Hamburg NM, Kinlay S, Lookstein R, Misra S, Mureebe L, Olin JW, Patel RAG, Regensteiner JG, Schanzer A, Shishehbor MH, Stewart KJ, Treat-Jacobson D, Walsh ME. 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2017; **69**: e71-e126 [PMID: 27851992 DOI: 10.1016/j.jacc.2016.11.007]

23 **Abola MTB**, Golledge J, Miyata T, Rha SW, Yan BP, Dy TC, Ganzon MSV, Handa PK, Harris S, Zhisheng J, Pinjala R, Robless PA, Yokoi H, Alajar EB, Bermudez-Delos Santos AA, Llanes EJB, Obrado-Nabablit GM, Pestaño NS, Punzalan FE, Tumanan-Mendoza B. Asia-Pacific Consensus Statement on the Management of Peripheral Artery Disease: A Report from the Asian Pacific Society of Atherosclerosis and Vascular Disease Asia-Pacific Peripheral Artery Disease Consensus Statement Project Committee. *J Atheroscler Thromb* 2020; **27**: 809-907 [PMID: 32624554 DOI: 10.5551/jat.53660]

24 **Golledge J**, Cronin O, Iyer V, Bradshaw B, Moxon JV, Cunningham MA. Body mass index is inversely associated with mortality in patients with peripheral vascular disease. *Atherosclerosis* 2013; **229**: 549-555 [PMID: 23742964 DOI: 10.1016/j.atherosclerosis.2013.04.030]

25 **Golledge J**, Ewels C, Muller R, Walker PJ. Association of chronic kidney disease categories defined with different formulae with major adverse events in patients with peripheral vascular disease. *Atherosclerosis* 2014; **232**: 289-297 [PMID: 24468141 DOI: 10.1016/j.atherosclerosis.2013.11.034]

26 **Parr A**, Buttner P, Shahzad A, Golledge J. Relation of infra-renal abdominal aortic calcific deposits and cardiovascular events in patients with peripheral artery disease. *Am J Cardiol* 2010; **105**: 895-899 [PMID: 20211340 DOI: 10.1016/j.amjcard.2009.10.067]

27 **Golledge J**, Jayalath R, Oliver L, Parr A, Schurgers L, Clancy P. Relationship between CT anthropometric measurements, adipokines and abdominal aortic calcification. *Atherosclerosis* 2008; **197**: 428-434 [PMID: 17675038 DOI: 10.1016/j.atherosclerosis.2007.06.027]

28 **Magee R**, Quigley F, McCann M, Buttner P, Golledge J. Growth and risk factors for expansion of dilated popliteal arteries. *Eur J Vasc Endovasc Surg* 2010; **39**: 606-611 [PMID: 20122854 DOI: 10.1016/j.ejvs.2009.12.031]

29 **Golledge J**, Quigley F, Velu R, Walker PJ, Moxon JV. Association of impaired fasting glucose, diabetes and their management with the presentation and outcome of peripheral artery disease: a cohort study. *Cardiovasc Diabetol* 2014; **13**: 147 [PMID: 25361884 DOI: 10.1186/s12933-014-0147-2]

30 **Harwood AE**, Smith GE, Cayton T, Broadbent E, Chetter IC. A Systematic Review of the Uptake and Adherence Rates to Supervised Exercise Programs in Patients with Intermittent Claudication. *Ann Vasc Surg* 2016; **34**: 280-289 [PMID: 27126713 DOI: 10.1016/j.avsg.2016.02.009]

31 **Lin E**, Nguyen CH, Thomas SG. Completion and adherence rates to exercise interventions in intermittent claudication: Traditional exercise *vs* alternative exercise - a systematic review. *Eur J Prev Cardiol* 2019; **26**: 1625-1633 [PMID: 31216860 DOI: 10.1177/2047487319846997]

32 **Moxon JV**, Ng E, Lazzaroni SM, Boult M, Velu R, Fitridge RA, Golledge J. Circulating biomarkers are not associated with endoleaks after endovascular repair of abdominal aortic aneurysms. *J Vasc Surg* 2018; **67**: 770-777 [PMID: 28843790 DOI: 10.1016/j.jvs.2017.06.090]

33 **Golledge J**, Leicht A, Crowther RG, Clancy P, Spinks WL, Quigley F. Association of obesity and metabolic syndrome with the severity and outcome of intermittent claudication. *J Vasc Surg* 2007; **45**: 40-46 [PMID: 17123770 DOI: 10.1016/j.jvs.2006.09.006]

34 **Morris DR**, Singh TP, Moxon JV, Smith A, Stewart F, Jones RE, Golledge J. Assessment and validation of a novel angiographic scoring system for peripheral artery disease. *Br J Surg* 2017; **104**: 544-554 [PMID: 28140457 DOI: 10.1002/bjs.10460]

35 **Moxon JV**, Jones RE, Wong G, Weir JM, Mellett NA, Kingwell BA, Meikle PJ, Golledge J. Baseline serum phosphatidylcholine plasmalogen concentrations are inversely associated with incident myocardial infarction in patients with mixed peripheral artery disease presentations. *Atherosclerosis* 2017; **263**: 301-308 [PMID: 28728066 DOI: 10.1016/j.atherosclerosis.2017.06.925]

36 **Queensland Health**. Queensland hospital admitted data collection manual 2015-2016. Available from: https://www.health.qld.gov.au/hsu/collections/qhapdc

37 **Peduzzi P**, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol* 1996; **49**: 1373-1379 [PMID: 8970487 DOI: 10.1016/s0895-4356(96)00236-3]

38 **Golledge J**, Fernando M, Lazzarini P, Najafi B, G Armstrong D. The Potential Role of Sensors, Wearables and Telehealth in the Remote Management of Diabetes-Related Foot Disease. *Sensors (Basel)* 2020; **20** [PMID: 32823514 DOI: 10.3390/s20164527]

39 **Saratzis A**, Jaspers NEM, Gwilym B, Thomas O, Tsui A, Lefroy R, Parks M, Htun V, Mera Z, Thatcher A, Bosanquet D, Forsythe R, Benson R, Dattani N, Dovell G, Lane T, Shalhoub J, Sidloff D, Visseren FLJ, Dorresteijn JAN, Richards T; Vascular and Endovascular Research Network (VERN) Collaborators. Observational study of the medical management of patients with peripheral artery disease. *Br J Surg* 2019; **106**: 1168-1177 [PMID: 31259387 DOI: 10.1002/bjs.11214]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 893-907

DOI: 10.4239/wjd.v12.i6.893

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Blood glucose response after oral lactulose intake in type 2 diabetic individuals

Thomas R Pieber, Eva Svehlikova, Ines Mursic, Tamara Esterl, Manfred Wargenau, Tina Sartorius, Lioba Pauly, Susann Schwejda-Guettes, Annalena Neumann, Valentin Faerber, John Friedrich Stover, Barbara Gaigg, Angelika Kuchinka-Koch

ORCID number: Thomas R Pieber 0000-0003-3554-0405; Eva Svehlikova 0000-0002-9743-2031; Ines Mursic 0000-0003-3836-6529; Tamara Esterl 0000-0002-2615-680X; Manfred Wargenau 0000-0002-0886-0158; Tina Sartorius 0000-0002-5264-7207; Lioba Pauly 0000-0002-2673-012X; Susann Schwejda-Guettes 0000-0002-6635-6005; Annalena Neumann 0000-0002-9716-329X; Valentin Faerber 0000-0003-4250-3389; John Friedrich Stover 0000-0001-9457-0941; Barbara Gaigg 0000-0002-8549-8877; Angelika Kuchinka-Koch 0000-0003-1874-1571.

Author contributions: Pieber TR, Svehlikova E, Mursic I, and Esterl T performed the study, data collection and interpretation; Stover JF, Pauly L, Schwejda-Guettes S, Kuchinka-Koch A, and Gaigg B designed and supervised the study; Wargenau M planned and performed the statistical analysis of the data and interpreted the results; Sartorius T, Neumann A, and Faerber V wrote the manuscript; all authors reviewed, edited, and approved the manuscript for submission.

Supported by Fresenius Kabi Deutschland GmbH, Germany.

Institutional review board

Thomas R Pieber, Eva Svehlikova, Ines Mursic, Tamara Esterl, Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Graz 8036, Austria

Manfred Wargenau, Department of Statistic, M.A.R.C.O. GmbH & Co. KG, Institute for Clinical Research and Statistics, Düsseldorf 40211, Germany

Tina Sartorius, Department of Nutritional CRO, BioTeSys GmbH, Esslingen 73728, Germany

Lioba Pauly, Susann Schwejda-Guettes, Department of Medical & Clinical Affairs, Market Access & Education Business Unit Enteral Nutrition, Bad Homburg 61352, Germany

Annalena Neumann, Valentin Faerber, Department of Medical, Clinical & Regulatory Affairs, Business Unit Parenteral Nutrition, Keto-Analogues and Standard I.V. Fluids, Fresenius Kabi Deutschland GmbH, Bad Homburg 61352, Germany

John Friedrich Stover, Department of Consultancy, Stover-Solutions, Zurich 8006, Switzerland

Barbara Gaigg, Angelika Kuchinka-Koch, Market Unit Lactulose, Fresenius Kabi Austria GmbH, Linz 4020, Austria

Corresponding author: Valentin Faerber, PhD, Director, Department of Medical, Clinical & Regulatory Affairs, Business Unit Parenteral Nutrition, Keto-Analogues and Standard I. V. Fluids, Fresenius Kabi Deutschland GmbH, Else-Kroener-Straße 1, Bad Homburg 61352, Germany. valentin.faerber@fresenius-kabi.com

Abstract

BACKGROUND

Lactulose is approved for the symptomatic treatment of constipation, a gastrointestinal (GI) complication common in individuals with diabetes. Lactulose products contain carbohydrate impurities (*e.g.*, lactose, fructose, galactose), which occur during the lactulose manufacturing process. These impurities may affect the blood glucose levels of individuals with type 2 diabetes mellitus (T2DM) using lactulose for the treatment of mild constipation. A previous study in healthy subjects revealed no increase in blood glucose levels after oral lactulose intake. However, it is still unclear whether the intake of lactulose increases blood glucose levels in individuals with diabetes.

statement: This study protocol was reviewed and approved by the Independent Ethics Committee of the Medical University of Graz, Austria.

Clinical trial registration statement: This study was registered in the European Union Drug Regulating Authorities Clinical Trials Database, No. 2018-002359-14.

Informed consent statement: All study participants provided written informed consent prior to enrollment.

Conflict-of-interest statement: The study was sponsored by Fresenius Kabi Deutschland GmbH, Germany. The sponsor's representatives contributed to the study design and the selection of outcome measures before study start but had no role in study execution or data collection, or data analyses. All other authors declare no potential conflicts of interest related to this paper.

Data sharing statement: No additional data are available.

CONSORT 2010 statement: The authors read the CONSORT 2010 Statement, and the manuscript was prepared and revised according to that statement.

Manuscript source: Unsolicited manuscript

Specialty type: Endocrinology and metabolism

AIM

To evaluate the blood glucose profile after oral lactulose intake in mildly constipated, non-insulin-dependent subjects with T2DM in an outpatient setting.

METHODS

This prospective, double-blind, randomized, controlled, single-center trial was conducted at the Clinical Research Center at the Medical University of Graz, Austria, in 24 adult Caucasian mildly constipated, non-insulin-dependent subjects with T2DM. Eligible subjects were randomized and assigned to one of six treatment sequences, each consisting of four treatments stratified by sex using an incomplete block design. Subjects received a single dose of 20 g or 30 g lactulose (crystal and liquid formulation), water as negative control or 30 g glucose as positive control. Capillary blood glucose concentrations were measured over a period of 180 min post dose. The primary endpoint was the baseline-corrected area under the curve of blood glucose concentrations over the complete assessment period [$AUC_{baseline\_c\ (0\text{-}180\ min)}$]. Quantitative comparisons were performed for both lactulose doses and formulations *vs* water for the equal lactulose dose *vs* glucose, as well as for liquid lactulose *vs* crystal lactulose. Safety parameters included GI tolerability, which was assessed at 180 min and 24 h post dose, and adverse events occurring up to 24 h post dose.

RESULTS

In 24 randomized and analyzed subjects blood glucose concentration-time curves after intake of 20 g and 30 g lactulose were almost identical to those after water intake for both lactulose formulations despite the different amounts of carbohydrate impurities (≤ 3.0% for crystals and approx. 30% for liquid). The primary endpoint [$AUC_{baseline\_c\ (0\text{-}180\ min)}$] was not significantly different between lactulose and water regardless of lactulose dose and formulation. Also with regard to all secondary endpoints lactulose formulations showed comparable results to water with one exception concerning maximum glucose level. A minor increase in maximum blood glucose was observed after the 30 g dose, liquid lactulose, in comparison to water with a mean treatment difference of 0.63 mmol/L (95% confidence intervals: 0.19, 1.07). Intake of 30 g glucose significantly increased all blood glucose endpoints *vs* 30 g liquid and crystal lactulose, respectively (all $P < 0.0001$). No differences in blood glucose response were observed between the different lactulose formulations. As expected, lactulose increased the number of bowel movements and was generally well tolerated. Subjects experienced only mild to moderate GI symptoms due to the laxative action of lactulose.

CONCLUSION

Blood glucose $AUC_{baseline\_c\ (0\text{-}180\ min)}$ levels in mildly constipated, non-insulin dependent subjects with T2DM are not affected by the carbohydrate impurities contained in 20 g and 30 g crystal or liquid lactulose formulations.

Key Words: Lactulose; Constipation; Blood glucose; Type 2 diabetes mellitus; Laxative; Sugar substitute

Core Tip: Individuals with diabetes are at risk of developing constipation, which can be symptomatically treated with lactulose. The question arose whether carbohydrate impurities in crystal and liquid lactulose formulations would increase blood glucose levels in individuals with diabetes. This study demonstrates that, at the recommended maintenance dosage of 20 g and at a higher dosage of 30 g lactulose, the blood glucose baseline-corrected area under the curve from 0 to 180 min levels in mildly constipated, non-insulin dependent subjects with type 2 diabetes mellitus are not affected.

Citation: Pieber TR, Svehlikova E, Mursic I, Esterl T, Wargenau M, Sartorius T, Pauly L, Schwejda-Guettes S, Neumann A, Faerber V, Stover JF, Gaigg B, Kuchinka-Koch A. Blood

Country/Territory of origin: Austria

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

Received: December 3, 2020
Peer-review started: December 3, 2020
First decision: March 14, 2021
Revised: April 7, 2021
Accepted: April 22, 2021
Article in press: April 22, 2021
Published online: June 15, 2021

P-Reviewer: Dahiya K, Firneisz G, García-Compeán D
S-Editor: Fan JR
L-Editor: A
P-Editor: Ma YJ

glucose response after oral lactulose intake in type 2 diabetic individuals. *World J Diabetes* 2021; 12(6): 893-907
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/893.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.893

INTRODUCTION

Disorders of the gastrointestinal (GI) tract are common in individuals with diabetes mellitus[1,2]. It is well known that persistent hyperglycemia negatively impacts enteric motor and sensory functions[3-6]. Diabetes-related dysmotility can be attributed to a loss of enteric neurons in the colon due to increased oxidative stress and apoptosis[7], leading to a variety of GI symptoms, including constipation, that severely reduce quality of life in individuals with diabetes[8].

Evidence-based treatment options for managing diabetes-related chronic constipation include lifestyle changes and furthermore, the use of laxatives such as the stimulants bisacodyl and senna glycoside and the osmotic agents polyethylene glycol (PEG), lactitol, and lactulose[9]. Lactulose is approved as a drug for the symptomatic treatment of constipation at a dose of 10 to 30 g and portal systemic encephalopathy at doses up to 100 g[10] and restores bowel movements by facilitating intestinal motility and secretion[11].

Lactulose is a disaccharide composed of galactose and fructose. It is neither absorbed in the small intestine nor digested by enzymes of the mammalian digestive tract. As an osmotic laxative, lactulose creates an osmotic gradient that increases the retention of water in the stool and subsequently enhances stool frequency, volume, and weight[12]. In addition, lactulose is completely metabolized by saccharolytic intestinal bacteria in the colon, thereby producing metabolites, *e.g.*, lactic acid, formic acid, and acetic acid, with osmotic abilities and peristalsis-stimulating effects[9,13]. Lactulose is known to enhance colonic transit time[14,15], which is reflected in the European Food Safety Authority-approved health claim that "lactulose contributes to an acceleration of intestinal transit"[16]. Furthermore, lactulose stimulates the growth or activity of a number of colonic bacteria referred to as bifidogenic effects[17] and is used as a prebiotic functional food ingredient.

Lactulose is produced by isomerization of the natural milk sugar lactose (galactose-glucose). During this process, carbohydrate impurities may arise and traces of the lactose may still be present in the final solution. Partial hydrolysis of lactulose can result in the formation of fructose and galactose. Tagatose can be formed by isomerization of galactose and epilactose by C2 epimerization of lactose. 3-Deoxyglyceropentuloses A and B may arise as by-products of the reaction. All these substances are listed in the European Pharmacopeia under 'related substances' and are denoted as "impurities" in the following)[18]. The amount and pattern of these impurities vary depending on the manufacturing process conditions. They can account for up to 3% carbohydrates in crystal lactulose and approx. 30% carbohydrates in liquid lactulose. After lactulose intake, these impurities may be absorbed in the digestive tract and thereby increase blood glucose levels. Theoretically, this may impact glycemic control in individuals with type 2 diabetes mellitus (T2DM). A previous study in healthy subjects showed no substantial increase in blood glucose after oral intake of 10 g and 20 g lactulose (crystals and liquid)[19]. These findings need to be confirmed in subjects with T2DM.

The aim of the present study was to investigate the potential impact of a single dose of 20 g or 30 g lactulose in currently marketed formulations (crystals and liquid) on blood glucose responses in mildly constipated, non-insulin-dependent subjects with T2DM in an outpatient setting.

MATERIALS AND METHODS

Study design

This was a prospective, double-blind, randomized, controlled, single-center trial with a four-period crossover and incomplete block design in subjects with mild functional constipation and T2DM. The study was conducted in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice and Austrian drug law and was approved by the Independent Ethics Committee of the Medical University of Graz,

Austria. All subjects gave written informed consent before any study-related activities were started. The study was registered in the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT No. 2018-002359-14).

The study was conducted at the Clinical Research Center at the Medical University of Graz, Austria, and consisted of a screening visit and four individual study visits separated by a washout period of 7 d (allowed range 4 to 14 d) to avoid carryover effects.

Randomization was performed by M.A.R.C.O. GmbH and Co. KG, Düsseldorf, Germany, in three blocks of six treatment sequences separately for each group stratified by sex, assigning random numbers 001 to 018 for female subjects and 021 to 038 for male subjects to treatment sequences. The randomization schedule was generated by a SAS® computer program based on the SAS® RANUNI function, which returns a random value from a uniform distribution. Subjects were assigned to random numbers in chronological order after enrollment to receive one of the six treatment sequences (Figure 1).

On the evening before each study visit, subjects were advised to eat a standardized dinner consisting of farmhouse bread with cream cheese and cucumber. Subjects were not allowed to consume food or drink other than water for at least 10 h before study product administration. On the morning of the study visits, subjects were instructed to drink one to two glasses of water (minimum 200 mL total) upon waking. Consumption of alcohol and intensive exercise were not allowed within 24 h before each study visit. Furthermore, the use of laxatives within 48 h before each study visit was prohibited. At each study visit, the administration of any antidiabetic agents was postponed to the end of the 180-min observation period to avoid interference with the blood glucose profile.

Study population

Eligible subjects were Caucasian men or women with non-insulin-dependent T2DM under stable antidiabetic treatment 3 mo prior to screening, treated with diet and oral antidiabetic agents (*e.g.*, metformin, alpha-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors, sulfonylurea, sodium-dependent glucose transporter 2 inhibitors, glucagon-like peptide-1 receptor agonists), and aged ≥ 18 and ≤ 75 years, who had glycosylated hemoglobin (HbA_{1c}) ≤ 7.5% and mild functional constipation according to the modified Rome IV criteria (approximately three to five bowel movements *per* week, of which one to two usually caused discomfort) during the previous 3 mo with symptom onset at least 6 mo before study start.

The main exclusion criteria were fasting capillary blood glucose levels < 4.4 mmol/L or > 10 mmol/L; body mass index (BMI) < 18.5 or ≥ 35 kg/m^2; change in body weight ≥ 10% within the last 3 mo; smoking habit; severe hepatic, renal, or cardiac disease; acute inflammatory bowel disease; GI obstruction or subocclusive syndrome; GI perforation or risk of GI perforation; abdominal pain of undetermined cause; major hospitalization or surgical event within the previous 3 mo; acute GI diseases including diarrhea and/or vomiting within the previous 2 wk; presence of disease or administration of medications/supplements other than antidiabetic treatment influencing digestion and absorption of carbohydrates or bowel habits; hereditary galactose or fructose intolerance; lactase deficiency or glucose-galactose malabsorption; intake of pre- or probiotics or medications known to affect glucose tolerance (*e.g.*, steroids, protease inhibitors, antipsychotics); and chronic administration of substances affecting blood coagulation which, in the investigator's opinion, would impact subject safety.

Sample size

For sample size estimation, a minimum blood glucose concentration difference of 0.6 mmol/L (corresponding to 30% of the theoretical maximum increase in blood glucose level after administration) between lactulose and water was considered. An effect size of 1 was defined for this trial. The power for detecting effect sizes of at least 1 was set to 90% at significance level α = 2.5% (one-sided). Based on this approach, 15 evaluable subjects would have been required for a complete crossover design assuming a correlation of 0.4. To obtain a balanced design, 16 subjects would have to be randomized. However, due to the incomplete block design with four periods for six treatments, a loss of efficiency of one-third was assumed. Therefore, 24 subjects with mild functional constipation and T2DM were planned to be randomized in the study.

Study products

Lactulose crystals (Laevolac®10 g powder for oral solution) and lactulose liquid

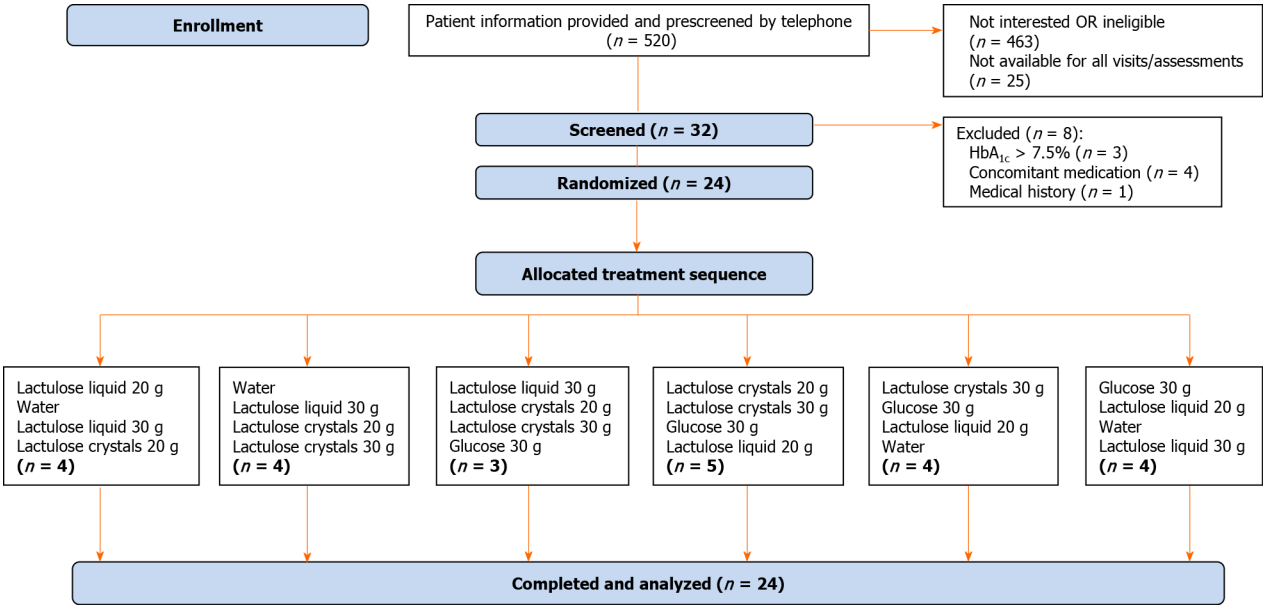

Figure 1 Flow chart of trial subjects. The main reasons for pre-screening failure included: Not interested (*n* = 85), missing signs of functional constipation (*n* = 220), insufficient blood glucose control (*n* = 24), not allowed concomitant medication, *e.g.*, insulin (*n* = 58), body mass index ≥ 35 kg/m^2 (*n* = 31) gastrointestinal or other relevant comorbidity (cancer, lactose intolerance *etc.*) (*n* = 39), smoking habit (*n* = 7). HbA_{1c}: Glycosylated hemoglobin.

(Laevolac® 10 g/15 mL oral solution) were produced by Fresenius Kabi Austria GmbH, Linz, Austria. The maximum carbohydrate impurities of both lactulose formulations according to the European Pharmacopoeia monographs were previously published[19]. The total impurities of the study products were within the threshold value of ≤ 3.0% in the crystal and approx. 30% in the liquid lactulose formulation. The effect on the blood glucose response was assessed at doses of 20 g and 30 g lactulose for both formulations and compared to still (non sparkling) water as a negative control and 30 g glucose (33 g glucose monohydrate powder, Roquette Frères, Lestrem, France) as a positive control. The study products were prepared and blinded on site by authorized unblinded study staff according to the randomization plan. Subjects as well as the investigator were blinded to the dosage of study products and the lactulose formulation. Lactulose and glucose were dissolved in 250 mL of still water and were provided as a single oral dose under the supervision of the study staff. The single dose had to be ingested within 5 min.

Data collection

Blood glucose concentration was measured in capillary whole blood obtained by finger stick according to ISO 26642 and analyzed using a HemoCue Glucose 201 RT Analyzer (HemoCue AB, Ängelholm, Sweden). Glucose was determined photometrically using a modified glucose dehydrogenase method. Blood glucose concentrations were assessed over a period of 180 min at defined time points (0, 15, 30, 45, 60, 90, 120, 150, and 180 min post-dose).

Data were transferred on a paper case report form to M.A.R.C.O. GmbH and Co. KG, Düsseldorf, Germany, for data management and statistical analysis.

Data analysis and statistics

For the primary endpoint, capillary blood glucose levels as baseline-corrected area under the curve from 0 to 180 min [$AUC_{baseline\_c\ (0\text{-}180\ min)}$] were determined, and quantitative comparisons after oral intake of lactulose products with water or glucose were performed. Secondary endpoints related to blood glucose concentrations were maximum blood glucose concentration (C_{max}), time to reach maximum concentration (T_{max}), maximum blood glucose concentration minus baseline value (Max_increase), $AUC_{(0\text{-}180\ min)}$, and incremental $AUC_{(0\text{-}180\ min)}$ [$iAUC_{(0\text{-}180\ min)}$] (*i.e.*, area above the baseline blood glucose concentration after oral intake of lactulose formulations or control products). An increase in blood glucose concentration ≥ 2.2 mmol/L is considered clinically relevant and is usually caused by an additional administration of 10 g carbohydrates, which might contribute to an increase in HbA_{1c} by 0.1% in the long term[20,21]. The following comparisons were made for both primary and secondary endpoints: (1) Both lactulose doses and formulations (20 g/30 g crystals/liquid) *vs*

water as a negative control; (2) 30 g lactulose (crystals/liquid) *vs* 30 g glucose as a positive control; and (3) Crystal lactulose *vs* liquid lactulose (for each dose 20 g/30 g). 95% two-sided confidence intervals (CIs) were calculated for all comparisons, which correspond to exploratory two-sided tests at 5% significance levels. In particular, if CIs did not include the threshold of clinical relevance, it could be concluded that lactulose has no clinically relevant impact on blood glucose levels.

GI tolerability was assessed at each study visit during the initial 180-min period and 24 h post dose using a 4-point Likert scale (none, mild, moderate or severe) to describe symptoms. The number of bowel movements was counted at each study visit until 24 h post dose for the different treatment groups. Consistency of stool was graded based on the Bristol Stool Form Scale (BSFS)[22] (type 1 = separate hard lumps, like nuts (hard to pass); type 2 = sausage-shaped but lumpy; type 3 = like a sausage with cracks on its surface; type 4 = like a sausage or snake, smooth and soft; type 5 = soft blobs with clear-cut edges, passed easily; type 6 = fluffy pieces with ragged edges, a mushy stool; type 7 = liquid, no solid pieces) with types 1 and 2 reflecting severe and mild constipation, types 3 to 5 showing normal stool, and types 6 and 7 reflecting mild and severe diarrhea.

Adverse events (AEs) were recorded in diaries over the entire study period after written informed consent was obtained. AEs were coded according to the latest Medical Dictionary for Regulatory Activities (version 22.0). The Common Terminology Criteria for Adverse Events (version 5.0) was used to assess the intensity of AEs. At each study visit, AEs were reviewed by the investigator and recorded in the case report form.

All parameters were listed and summarized with descriptive statistics (n = number of no-missing values, arithmetic mean, standard deviation, minimum, median, maximum, first and third quartiles) or frequency tables by treatment, as appropriate.

The primary endpoint, untransformed $AUC_{baseline\_c\ (0\text{-}180\ min),}$ was analyzed using a mixed analysis-of-variance model with sex (as between-subject fixed effect), treatment (six levels), period (four levels) and baseline blood glucose level to adjust for potential inter- and intraindividual differences in baseline blood glucose according to the study period as fixed effect and subject as random effect. No further covariates were considered. Least square (LS) means including 95%CIs were calculated for all treatments and treatment differences. Secondary endpoints were evaluated analogously to the primary endpoint. Raw data listings, summary tables and inferential analyses were carried out using SAS® software (version 9.3).

Data are presented for the intention-to-treat population, which was identical to the per-protocol population in this study. Exploration of possible carryover effects was not obligatory due to the 7-d (allowed range 4 to 14 d) washout period.

RESULTS

A total of 32 subjects were screened, and 24 subjects were enrolled from November 2018 to March 2019. Demographic and baseline data of randomized subjects are summarized in Table 1. Overall, 16 subjects (66.7%) were male, and eight subjects (33.3%) were female. The treatment sequence groups were comparable to the subject's baseline age, height, weight, and BMI. The mean baseline values of fasting blood glucose ranged from 6.9 mmol/L to 7.3 mmol/L. Before randomization, 75% of the subjects reported bowel symptoms, and 67% had constipation for more than one year. The average number of bowel movements *per* week was three to five movements, and almost all subjects (n = 22) had an average of one to two bowel movements with discomfort *per* week prior to randomization. Only two patients had three to five bowel movements with discomfort *per* week. Only two subjects used laxatives to encourage defecation before randomization. However, these subjects abstained from using laxatives two days before and up to 24 h after the respective study visits.

Four sequence groups achieved the anticipated size of four subjects, whereas one more subject was allocated to one sequence group (n = 5) and one less to another sequence group (n = 3) (Figure 1). This slight imbalance did not constitute a protocol deviation. All 24 subjects were treated according to the randomization schedule and successfully completed the study without any major protocol deviations.

Lactulose vs negative control (water)

The primary endpoint $AUC_{baseline\_c\ (0\text{-}180\ min)}$ did not significantly differ between lactulose and water intake, regardless of lactulose dose and formulation. The estimated LS means for $AUC_{baseline\_c\ (0\text{-}180\ min)}$ of all lactulose doses and formulations ranged from -30.70

Table 1 Demographic and baseline data of subjects (*n* = 24)

Variable	mean	SD	Min	Median	Max
Age (yr)	62.2	7.61	45	62.5	73
BMI (kg/m^2)	30.0	3.0	23.3	30.1	34.7
Systolic BP (mmHg)	142.8	19.1	114.0	141.5	196.0
Diastolic BP (mmHg)	88.1	9.1	73.0	90.0	105.0
HbA_{1c} (%)	6.6	2.6	5.5	6.6	7.5

BMI: Body mass index; BP: Blood pressure; HbA_{1c}: Glycosylated hemoglobin; Max: Maximum; Min: Minimum, SD: Standard deviation.

to -54.40 min/mmol/L. The mean $AUC_{baseline\_c\ (0\text{-}180\ min)}$ after water intake was -53.21 min/mmol/L (95%CI: -99.14, -7.28). This implies a net decrease in blood glucose concentration over time after lactulose intake compared to the respective baseline blood glucose level. The average net decrease over the assessment period, calculated as $AUC_{baseline\_c\ (0\text{-}180\ min)}$/180 min, was approx. -0.3 mmol/L.

Mean blood glucose concentration-time curves after intake of 20 g (Table 2) or 30 g (Table 3) crystal lactulose did not differ from the mean blood glucose concentration-time curve after intake of water (Figure 2). The mean blood glucose concentration-time curve for 20 g liquid lactulose was also comparable to that of water (Table 4). The maximum blood glucose concentrations appeared slightly higher after intake of the 30 g liquid lactulose compared to water, showing mean maximum increases of 1.00 mmol/L and 0.37 mmol/L after intake of 30 g liquid lactulose and water, respectively (Table 5). Thus, the mean maximum increase after 30 g liquid lactulose was 0.63 mmol/L (95%CI: 0.19, 1.07) (P = 0.0059) higher than that after water. The median (range) T_{max} was 30 min (0 to 60 min) after 30 g liquid lactulose intake and 22.5 min (0 to 150 min) after the intake of water.

Lactulose vs positive control (30 g glucose)

A glucose dose of 30 g was expected to induce higher blood glucose concentrations than 30 g of lactulose. Indeed, significant differences (P < 0.0001) in all study endpoints were observed between glucose and both lactulose formulations (Table 6). The mean $AUC_{baseline\_c\ (0\text{-}180\ min)}$ of 460 min/mmol/L for glucose and especially the means of -41 min/mmol/L and -31 min/mmol/L for 30 g lactulose formulations (*i.e.*, even slightly lowered glucose levels) demonstrated no effect of lactulose intake on blood glucose levels.

Likewise, $iAUC_{(0\text{-}180\ min)}$, $AUC_{(0\text{-}180\ min)}$, C_{max} and maximum increase were significantly lower for 30 g of both lactulose formulations compared to 30 g glucose.

As expected for subjects with T2DM, a pronounced increase in blood glucose concentration to 13.2 mmol/L with a median T_{max} of 60 min was observed after glucose administration. Blood glucose returned to nearly baseline levels after 180 min without any use of antidiabetic agents.

Crystal vs liquid lactulose

No noticeable differences in blood glucose response were observed between the different lactulose formulations. After 20 g lactulose intake, the LS mean $AUC_{baseline\_c\ (0\text{-}180\ min)}$ was -54.40 min/mmol/L (95%CI: -100.23, -8.57) and -30.72 min/mmol/L (95%CI: -75.26, 13.83) for the crystal and liquid formulations, respectively (P = 0.4218). After 30 g lactulose intake, the LS mean $AUC_{baseline\_c\ (0\text{-}180\ min)}$ values were -30.70 min/mmol/L (95%CI: -76.75, 15.35) and -41.01 min/mmol/L (95% CI: -88.72, 6.70) for the crystal and liquid formulations, respectively, (P = 0.7379). The $AUC_{(0\text{-}180\ min)}$ and $iAUC_{(0\text{-}180\ min)}$ results were similar for both formulations (Tables 2-6). For the different lactulose formulations, the mean C_{max} was 7.50 and 7.77 mmol/L for 20 g and 30 g crystal lactulose, respectively, and 7.75 and 8.07 mmol/L for 20 g and 30 g liquid lactulose, respectively. The median T_{max} was similar for all lactulose formulations and doses (T_{max} data not shown).

Bowel movements and consistency

Overall, the total number of bowel movements was greater after lactulose intake regardless of dose, formulation, or time period (from 0 to ≤ 3 h or from > 3 to ≤ 24 h) compared to the control groups and occurred more frequently between > 3 to ≤ 24 h

Table 2 Blood glucose endpoints: 20 g crystal lactulose *vs* water

	20 g crystal lactulose		Water		Treatment	*P* value
Variable	***n***	**LS mean (95%CI)**	***n***	**LS mean (95%CI)**	**Difference (95%CI)**	
$AUC_{baseline\_c\ (0\text{-}180\ min)}$ (min/mmol/L)	16	-54.40 (-100.23, -8.57)	16	-53.21 (-99.14, -7.28)	-1.20 (-60.78, 58.39)	0.9682
$AUC_{(0\text{-}180\ min)}$ (min/mmol/L)	16	1218.33 (1172.50, 1264.16)	16	1219.53 (1173.60, 1265.46)	-1.20 (-60.78, 58.39)	0.9682
$iAUC_{(0\text{-}180\ min)}$ (min/mmol/L)	16	17.00 (-14.43, 48.43)	16	18.44 (-13.04, 49.92)	-1.44 (-45.16, 42.29)	0.9478
C_{max} (mmol/L)	16	7.50 (7.17, 7.82)	16	7.44 (7.11, 7.77)	0.06 (-0.38, 0.50)	0.7999
Max_increase (mmol/L)	16	0.43 (0.10, 0.75)	16	0.37 (0.04, 0.70)	0.06 (-0.38, 0.50)	0.7999

$AUC_{baseline\_c\ (0\text{-}180\ min)}$: Baseline-corrected area under the curve from 0 to 180 min; $AUC_{(0\text{-}180\ min)}$: Area under the curve from 0 to 180 min; $iAUC_{(0\text{-}180\ min)}$: Incremental $AUC_{(0\text{-}180\ min)}$, *i.e.*, area above baseline levels of blood glucose concentration; C_{max}: Maximum blood glucose concentration; Max_increase: Maximum blood glucose concentration minus baseline value; LS mean: Adjusted least square mean obtained from analysis of covariance model.

Table 3 Blood glucose endpoints: 30 g crystal lactulose *vs* water

	30 g crystal lactulose		Water		Treatment	*P* value
Variable	***n***	**LS mean (95%CI)**	***n***	**LS mean (95%CI)**	**Difference (95%CI)**	
$AUC_{baseline\_c\ (0\text{-}180\ min)}$ (min/mmol/L)	16	-30.70 (-76.75, 15.35)	16	-53.21 (-99.14, -7.28)	22.51 (-37.05, 82.06)	0.4529
$AUC_{(0\text{-}180\ min)}$ (min/mmol/L)	16	1242.03 (1195.98, 1288.08)	16	1219.53 (1173.60, 1265.46)	22.51 (-37.05, 82.06)	0.4529
$iAUC_{(0\text{-}180\ min)}$ (min/mmol/L)	16	27.65 (-3.91, 59.21)	16	18.44 (-13.04, 49.92)	9.21 (-34.39, 52.81)	0.6744
C_{max} (mmol/L)	16	7.77 (7.44, 8.09)	16	7.44 (7.11, 7.77)	0.33 (-0.11, 0.76)	0.1426
Max_increase (mmol/L)	16	0.70 (0.37, 1.02)	16	0.37 (0.04, 0.70)	0.33 (-0.11, 0.76)	0.1426

$AUC_{baseline\_c\ (0\text{-}180\ min)}$: Baseline-corrected area under the curve from 0 to 180 min; $AUC_{(0\text{-}180\ min)}$: Area under the curve from 0 to 180 min; $iAUC_{(0\text{-}180\ min)}$: Incremental $AUC_{(0\text{-}180\ min)}$, *i.e.*, area above baseline levels of blood glucose concentration; C_{max}: Maximum blood glucose concentration; Max_increase: Maximum blood glucose concentration minus baseline value; LS mean: Adjusted least square mean obtained from analysis of covariance model.

Table 4 Blood glucose endpoints: 20 g liquid lactulose *vs* water

	20 g liquid lactulose		Water		Treatment	*P* value
Variable	***n***	**LS mean (95%CI)**	***n***	**LS mean (95%CI)**	**Difference (95%CI)**	
$AUC_{baseline\_c\ (0\text{-}180\ min)}$ (min/mmol/L)	17	-30.72 (-75.26, 13.83)	16	-53.21 (-99.14, -7.28)	22.49 (-35.18, 80.16)	0.4387
$AUC_{(0\text{-}180\ min)}$ (min/mmol/L)	17	1242.02 (1197.47, 1286.56)	16	1219.53 (1173.60, 1265.46)	22.49 (-35.18, 80.16)	0.4387
$iAUC_{(0\text{-}180\ min)}$ (min/mmol/L)	17	34.42 (3.98, 64.86)	16	18.44 (-13.04, 49.92)	15.98 (26.78, 58.75)	0.4578
C_{max} (mmol/L)	17	7.747 (7.43, 8.06)	16	7.44 (7.11, 7.77)	0.31 (-0.12, 0.73)	0.1560
Max_increase (mmol/L)	17	0.68 (0.36, 0.99)	16	0.37 (0.043, 0.70)	0.31 (-0.12, 0.73)	0.1560

$AUC_{baseline\_c\ (0\text{-}180\ min)}$: Baseline-corrected area under the curve from 0 to 180 min; $AUC_{(0\text{-}180\ min)}$: Area under the curve from 0 to 180 min; $iAUC_{(0\text{-}180\ min)}$: Incremental $AUC_{(0\text{-}180\ min)}$, *i.e.*, area above baseline levels of blood glucose concentration; C_{max}: Maximum blood glucose concentration; Max_increase: Maximum blood glucose concentration minus baseline value; LS mean: Adjusted least square mean obtained from analysis of covariance model

after study product administration (Figure 3). As expected, 88% (20 g crystal lactulose), 100% (30 g crystal lactulose), 94% (20 g liquid lactulose), and 93% (30 g liquid lactulose) of subjects had at least 1 bowel movement during the first 24 h after lactulose intake compared to 63% and 69% of subjects after intake of water and 30 g glucose, respectively.

Table 5 Blood glucose endpoints: 30 g liquid lactulose *vs* water

	30 g liquid lactulose		Water		Treatment	*P* value
Variable	***n***	**LS mean (95%CI)**	***n***	**LS mean (95%CI)**	**Difference (95%CI)**	
$AUC_{baseline\_c\ (0\text{-}180\ min)}$ (min/mmol/L)	15	-41.01 (-88.72, 6.70)	16	-53.21 (-99.14, -7.28)	12.20 (-47.48, 71.88)	0.6843
$AUC_{(0\text{-}180\ min)}$ (min/mmol/L)	15	1231.73 (1184.02, 1279.43)	16	1219.53 (1173.60, 1265.46)	12.20 (-47.48, 71.88)	0.6843
$iAUC_{(0\text{-}180\ min)}$ (min/mmol/L)	15	42.05 (9.51, 74.60)	16	18.44 (-13.04, 49.92)	23.61 (-20.52, 67.75)	0.2890
C_{max} (mmol/L)	15	8.07 (7.73, 8.41)	16	7.44 (7.11, 7.77)	0.63 (0.19, 1.07)	0.0059
Max_increase (mmol/L)	15	1.00 (0.66, 1.34)	16	0.37 (0.043, 0.70)	0.63 (0.19, 1.07)	0.0059

$AUC_{baseline\_c\ (0\text{-}180\ min)}$: Baseline-corrected area under the curve from 0 to 180 min; $AUC_{(0\text{-}180\ min)}$: Area under the curve from 0 to 180 min; $iAUC_{(0\text{-}180\ min)}$: Incremental $AUC_{(0\text{-}180\ min)}$, *i.e.*, area above baseline levels of blood glucose concentration; C_{max}: Maximum blood glucose concentration; Max_increase: Maximum blood glucose concentration minus baseline value; LS mean: Adjusted least square mean obtained from analysis of covariance model

Table 6 Blood glucose endpoints: 30 g glucose vs 30 g liquid and crystal lactulose

	Glucose 30 g		Liquid and crystal lactulose 30 g			Treatment	*P* value
Variable	***n***	**LS mean (95%CI)**	**Formu-lation**	***n***	**LS mean (95%CI)**	**Difference (95%CI)**	
$AUC_{baseline\_c\ (0\text{-}180\ min)}$ (min/mmol/L)	16	459.83 (413.74, 505.92)	Liquid	15	-41.01 (-88.72, 6.70)	500.84 (439.43, 562.26)	< 0.0001
			Crystals	16	-30.70 (-76.75, 15.35)	490.54 (431.89, 549.18)	< 0.0001
$AUC_{(0\text{-}180\ min)}$ (min/mmol/L)	16	1732.57 (1686.48, 1778.66)	Liquid	15	1231.73 (1184.02, 1279.43)	500.84 (439.43, 562.26)	< 0.0001
			Crystals	16	1242.03 (1195.98, 1288.08)	490.54 (431.89, 549.18)	< 0.0001
$iAUC_{(0\text{-}180\ min)}$ (min/mmol/L)	16	481.14 (449.56, 512.72)	Liquid	15	42.05 (9.51, 74.60)	439.08 (394.55, 483.62)	< 0.0001
			Crystals	16	27.65 (-3.91, 59.21)	453.49 (409.96, 497.02)	< 0.0001
C_{max} (mmol/L)	16	13.22 (12.90, 13.55)	Liquid	15	8.07 (7.73, 8.41)	5.16 (4.71, 5.61)	< 0.0001
			Crystals	16	7.77 (7.44, 8.09)	5.46 (5.02, 5.89)	< 0.0001
Max_increase (mmol/L)	16	6.15 (5.83, 6.48)	Liquid	15	1.00 (0.66, 1.34)	5.16 (4.71, 5.61)	< 0.0001
			Crystals	16	0.70 (0.37, 1.02)	5.46 (5.02, 5.89)	< 0.0001

$AUC_{baseline\_c\ (0\text{-}180\ min)}$: Baseline-corrected area under the curve from 0 to 180 min; $AUC_{(0\text{-}180\ min)}$: Area under the curve from 0 to 180 min; $iAUC_{(0\text{-}180\ min)}$: Incremental $AUC_{(0\text{-}180\ min)}$, *i.e.*, area above baseline levels of blood glucose concentration; C_{max}: Maximum blood glucose concentration; Max_increase: Maximum blood glucose concentration minus baseline value; LS mean: Adjusted least square mean obtained from analysis of covariance model.

On average, the number of bowel movements was higher in the lactulose groups [20 g crystal lactulose: 1.75 ± 1.39 (mean ± SD), range 0-5; 30 g crystal lactulose: 2.06 ± 1.00, range 1-4; 20 g liquid lactulose: 1.88 ± 1.05, range 0-4; 30 g liquid lactulose: 2.00 ± 1.25, range 0-4] compared to water (0.81 ± 0.83, range 0-3) and 30 g glucose (0.94 ± 0.77, range 0-2).

Constipation, expressed by BSFS types 1 and 2, was observed more often in subjects receiving 30 g glucose (25%) compared to 20 g crystal lactulose (13%), 30 g crystal lactulose (6%), 20 g liquid lactulose (18%), 30 g liquid lactulose (20%), and water (0%). Constipation did not lead to a discontinuation of participation in the study.

Normal stool, reflected by BSFS types 3-5, was present more often in subjects receiving lactulose (20 g crystal lactulose: 81%; 30 g crystal lactulose: 88%; 20 g liquid lactulose: 88%; 30 g liquid lactulose: 67%) compared to water (69%) and 30 g glucose (56%).

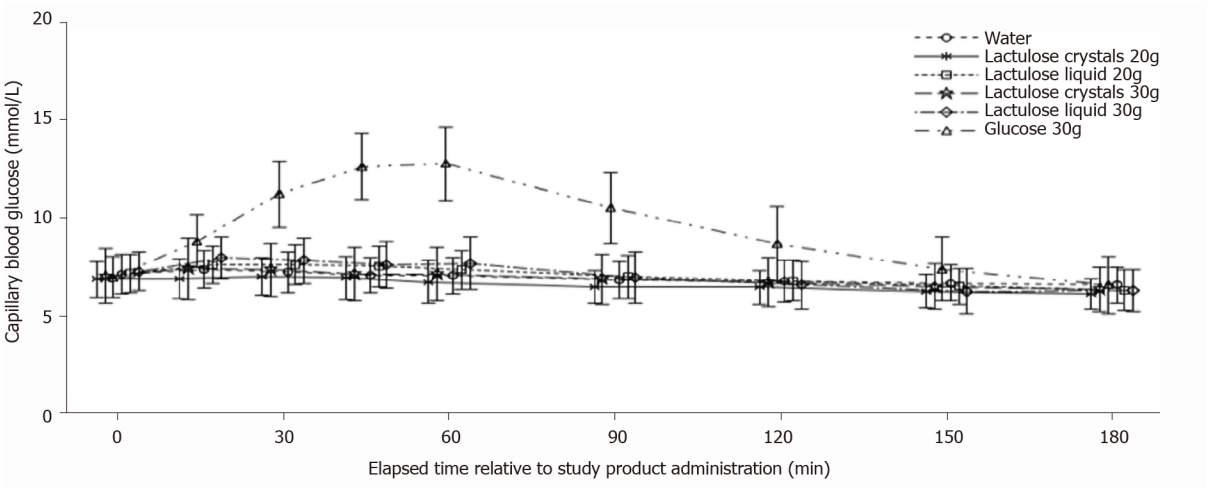

Figure 2 Blood glucose concentration-time curves. Each line represents mean ± SD. *n* = 16 each for water, 20 g crystal lactulose, 30 g crystal lactulose, and 30 g glucose, *n* = 17 for 20 g liquid lactulose, *n* = 15 for 30 g liquid lactulose.

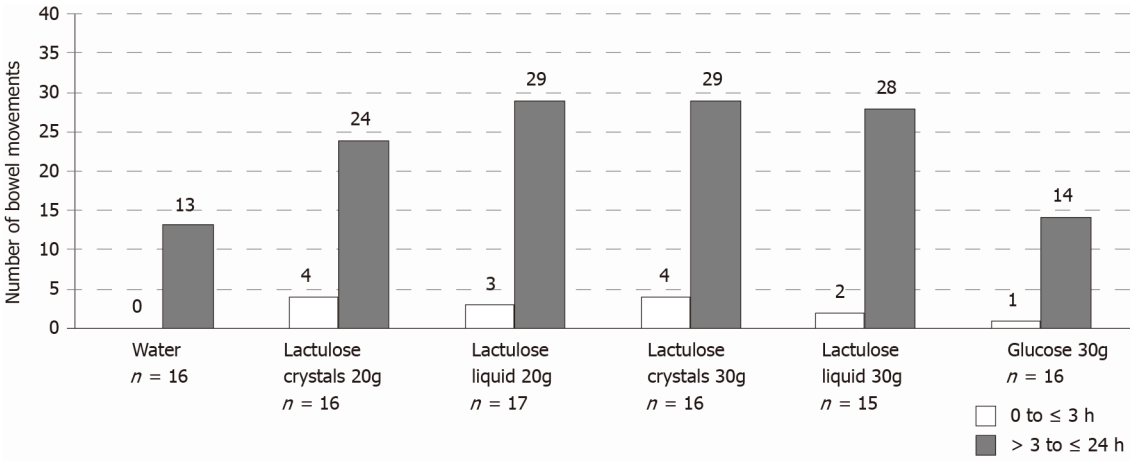

Figure 3 Cumulative numbers of bowel movements *per* treatment and time after oral intake of water, glucose, or lactulose for all subjects.

Diarrhea, expressed by BSFS types 6 and 7, was observed more often in subjects receiving lactulose (20 g crystal lactulose: 44%; 30 g crystal lactulose: 56%; 20 g liquid lactulose: 59%; 30 g liquid lactulose: 80%) compared to water (13%) and 30 g glucose (13%). Diarrhea did not lead to a discontinuation of participation in the study.

AEs and GI tolerability

All 24 subjects experienced at least one AE: 12 (75.0%) subjects after intake of 20 g crystal lactulose, 14 (87.5%) subjects after intake of 30 g crystal lactulose, 15 (88.2%) subjects after intake of 20 g liquid lactulose, 15 (100%) subjects after intake of 30 g liquid lactulose, seven (43.8%) subjects after intake of water, and nine (56.3%) subjects after intake of 30 g glucose. Of note, the reported AEs mainly affected the digestive system with mild to moderate abdominal distension, diarrhea, flatulence, and abnormal GI sounds. Moderate AEs, such as rumbling or abdominal pain, were reported more frequently after intake of the 30 g liquid lactulose. Overall, none of the AEs were serious, and no AE led to study discontinuation or modification of the study product dosage.

Distension and flatulence of mild to severe intensity occurred in 36% and 52% of subjects after lactulose intake (> 3 to ≤ 24 h post-dose), respectively. For comparison, distension was reported in 19% of subjects in the water group and 25% of subjects in the glucose group, and flatulence was reported in 31% of subjects in the water group and 25% of subjects in the glucose group.

Overall, all study products were well tolerated. Tolerability 3 h after intake was assessed as "very good" (100% of subjects in the 20 g crystal lactulose, water, and 30 g glucose, 81% in the 30 g crystal lactulose, 94% in the 20 g liquid lactulose, and 93% in the 30 g liquid lactulose group) and "good" (19% in the 30 g crystal lactulose, 6% in the 20 g liquid lactulose, and 7% in the 30 g liquid lactulose group). Similar tolerability was reported up to 24 h post-dose ("very good" in 47%-94% of subjects, "good" in 6%-53% of subjects). Only in the 30 g lactulose groups did some subjects also report "moderate" GI tolerability (6% and 13% of subjects in the 30 g crystal and liquid lactulose groups, respectively). No subjects reported "poor" GI tolerability through 24 h after intake of study products.

DISCUSSION

The present study tested the hypothesis that single oral doses of 20 g and 30 g of crystal and liquid lactulose have no clinically relevant impact on blood glucose levels in mildly constipated, non-insulin-dependent subjects with T2DM. The study was designed as a prospective, double-blind, randomized trial with a four-period crossover and incomplete block design. Compared to a previous study in healthy subjects that used doses of 10 g and 20 g[19], a higher lactulose dose (30 g) was used to address potential safety concerns regarding "impurity load" in subjects with T2DM. However, with respect to the amount of carbohydrate impurities (up to 3% for crystal lactulose and approx. 30% for liquid lactulose), only minor effects on blood glucose concentrations were expected. Another study objective was to compare the two lactulose formulations in terms of blood glucose concentration-time responses.

According to the prescribing information, the recommended maintenance dosage range of lactulose in adults with chronic constipation is 10-20 g *per* day, both for crystal and liquid formulations. The higher dose of 30 g *per* day can be indicated as a starter dose, to achieve an immediate laxative effect. Crystal and liquid lactulose at doses of 20 g as well as crystal lactulose at a dose of 30 g did not affect any measures of blood glucose response in mildly constipated, non-insulin dependent subjects with T2DM when compared to water, whether expressed as $AUC_{baseline\_c\ (0\text{-}180\ min)}$, C_{max} or maximum increase. Merely after the intake of 30 g liquid lactulose, a small significant increase in calculated blood glucose parameters C_{max} and maximum increase compared to water (negative control) was observed. However, in the interpretation of this result, it should be taken into account that maximum increase is a secondary endpoint in our study and is solely based on a single sampling point and calculation. Furthermore, individual glucose profiles showed a rather heterogenic pattern with maximum values occurring at different times ranging between baseline and 180 min (as a second peak) after administration. Thus, this observation presumably appeared due to random variability and is unlikely to be induced by 30 g liquid lactulose. The observed result is clinically not relevant, since the upper limit of the CI is clearly below the 2.2 mmol/L threshold of clinical relevance and the $AUC_{baseline\_c\ (0\text{-}180\ min)}$ was comparable to that of water.

These findings are in agreement with previous studies demonstrating that a 25 g lactulose dose did not affect blood glucose levels in female lactose digesters and maldigesters[23] or in individuals with diabetes[24]. There is only one case report referring to higher blood glucose levels after changing the lactulose syrup brand in a subject with diet-controlled diabetes and cirrhosis[25]. It is notable that the carbohydrate impurity amount and pattern in lactulose products vary depending on the manufacturing process conditions. A different brand may, therefore, have a higher content of impurities, which may have been the reason for the increase in blood glucose levels described in this case report.

The oral intake of unabsorbable disaccharides may affect carbohydrate metabolism by reducing transit time and, possibly, glucose absorption[25,26]. The intake of both the 20 g and 30 g lactulose doses, regardless of the formulation, resulted in a slight net decrease in blood glucose concentrations of approx. -0.3 mmol/L from baseline as assessed by an overall negative $AUC_{baseline\_c\ (0\text{-}180\ min)}$. This decrease, however, was within the normal physiological range of fasting blood glucose and comparable to what was observed after intake of water. Lactulose-induced impairment of intestinal carbohydrate uptake and carbohydrate metabolism was not observed under fasting conditions. The blood glucose concentrations remained largely stable despite a continuous fasting period for 3 h after oral intake of lactulose. Therefore, there is no risk for hypoglycemia after oral lactulose intake in individuals with T2DM.

With regard to safety and tolerability, the GI symptoms experienced by the participating subjects after single oral lactulose intake are well known. The reported

AEs included diarrhea, flatulence, and abdominal discomfort that, as expected, were reported more frequently after intake of the higher lactulose dose. Usually, GI symptoms disappear after some days of lactulose treatment. Most treatment-emergent AEs were mild to moderate in severity, considered to be related to the study treatment, and resolved by the end of the 24 h posttreatment observation period. Overall, lactulose was well tolerated, and no unexpected safety issues were identified.

Both lactulose formulations and doses showed the desired laxative action by increasing bowel movements compared to the control treatments predominantly between > 3 to ≤ 24 h after intake.

It has been demonstrated that individuals with diabetes experience a decrease in gut microbial diversity with an increase in opportunistic pathogens[9]. In contrast to other laxatives, lactulose is metabolized by gut bacteria, thereby contributing to the maintenance or development of a healthy colonic microbiota. Lactulose is used as an energy source by bifidobacteria and lactobacilli in the colon, allowing lactulose to be regarded as a prebiotic agent[9]. In a prospective, randomized, controlled trial in 65 chronically constipated nondiabetic adults who received PEG-4000 or lactulose over 4 wk, fecal bifidobacterial counts were higher in the lactulose group than in the PEG group (P = 0.04)[17]. Other types of laxatives (*e.g.*, bulk-forming laxatives such as psyllium and methylcellulose, other osmotic laxatives such as sorbitol and PEG, or stimulant agents such as senna glycoside and bisacodyl) may have further disadvantages. Specifically, bulk-forming laxatives may interfere with the absorption of medications commonly prescribed for use by older subjects (*e.g.*, warfarin, aspirin, iron, calcium)[27-29], while stimulant laxatives such as bisacodyl indicated only for short-term use may induce dehydration and loss of electrolytes[28]. PEG may cause anaphylaxis[9] with a contraindication of use in severe inflammatory conditions of the intestinal tract (*e.g.*, ulcerative colitis, Crohn's disease, toxic megacolon) or intestinal perforation[9].

Our study confirms that based on the $AUC_{baseline\_c\ (0\text{-}180\ min)}$ the recommended maintenance doses of 20 g and the higher dose of 30 g lactulose (crystals or liquid) can be used in mildly constipated, non-insulin dependent subjects with T2DM. These individuals may particularly benefit from the prebiotic effect of this laxative without experiencing an impact on blood glucose levels and glycemic management.

The present study has several strengths and limitations. First, an obvious strength is that the study was conducted in a relatively short time period, with high reliability and power. Second, the intention-to-treat population was identical to the per-protocol population in this study.

One limitation of the current study is that subjects may have distinguished between water and the other study products due to the slightly sweet taste of lactulose and glucose. Although subjects were blinded to both the dose and formulation of lactulose, as well as both control products, it was not feasible to ensure an identical taste of all study products. However, placebo effects on blood glucose concentration were not identified in a previous study in healthy subjects[19]. Therefore, a potential impact of this confounding factor on the blood glucose response is not expected. Adherence of subjects to the pre-visit restrictions was verified using diaries and questionnaires that were checked by the investigator at the start of each study visit. In case of noncompliance, the study visit was to be postponed. Thus, the potential bias is considered negligible. All lactulose doses and formulations were only tested in a single oral dose. During the study, 16 participants received three different lactulose doses, while 8 participants received two different lactulose doses. We assume that repeated daily doses will unlikely impact blood glucose levels if single doses do not increase blood glucose levels.

Eventually, applying the listed inclusion and exclusion criteria, the study population consisted exclusively of outpatients with T2DM and mild constipation without any endocrine or GI comorbidities. Since our aim was to specifically investigate the effect of lactulose on blood sugar response, we defined these criteria to ensure that any confounders masking the potential effects of lactulose, such as medications or comorbidities, can be ruled out. In fact, it is common practice to define strict inclusion/exclusion criteria for clinical studies to minimize the influence of potential confounders and achieve a certain degree of homogeneity. We consider the study population to be representative for the patient group who may benefit from lactulose administration.

CONCLUSION

In conclusion, the present study demonstrates that, at the recommended maintenance dose of 20 g and at the higher dose of 30 g lactulose, the blood glucose $AUC_{baseline\_c\ (0\text{-}180\ min)}$ levels in mildly constipated, non-insulin dependent subjects with T2DM are not affected by the carbohydrate impurities contained in crystal or liquid lactulose formulations. Lactulose increased the number of bowel movements with only mild to moderate known GI side effects.

ARTICLE HIGHLIGHTS

Research background

Lactulose is approved for the symptomatic treatment of constipation, a gastrointestinal (GI) complication common in individuals with diabetes. Lactulose products contain carbohydrate impurities that occur during the lactulose manufacturing process. These impurities may affect the blood glucose levels of individuals with type 2 diabetes mellitus (T2DM) using lactulose for the treatment of mild constipation.

Research motivation

Currently, there is no information on whether lactulose in marketed formulations (crystals and liquid) has an impact on the blood glucose profile in mildly constipated, non-insulin-dependent subjects with T2DM.

Research objectives

The main objective was to assess possible changes in blood glucose levels after oral intake of lactulose in mildly constipated, non-insulin-dependent subjects with T2DM in an outpatient setting.

Research methods

The study was performed as a prospective, double-blind, randomized, controlled, single-center trial with a four-period crossover and incomplete block design in a total of 24 mildly constipated non-insulin-dependent subjects with T2DM. Capillary blood glucose concentrations were assessed over a period of 180 min after a single oral dose of 20 g or 30 g lactulose (crystal and liquid formulation). Water and 30 g glucose served as a negative and positive control, respectively.

Research results

Lactulose when administered at the recommended maintenance dose of 20 g and at a higher dose of 30 g (crystal or liquid formulation) had no impact on blood glucose baseline-corrected area under the curve of blood glucose concentrations over the complete assessment period [$AUC_{baseline\_c\ (0\text{-}180\ min)}$]. The early, small, self-limited increase in maximal blood glucose increase of 0.63 mmol/L (maximum blood glucose concentration, $P = 0.0059$ *vs* water) compared to water is not clinically relevant. As expected for subjects with T2DM, the dose of 30 g glucose (positive control) resulted in a pronounced increase in blood glucose concentration. No differences in blood glucose response were observed between the different lactulose formulations. Lactulose increased the number of bowel movements and was generally well tolerated with only mild to moderate GI symptoms due to the laxative action of lactulose.

Research conclusions

As expressed by the $AUC_{baseline\_c\ (0\text{-}180\ min)}$ carbohydrate impurities in oral lactulose products administered at the recommended doses of 20 g/d and 30 g/d do not have to be considered for the blood glucose management of mildly constipated, non-insulin-dependent individuals with T2DM taking lactulose as a laxative.

Research perspectives

Future research could focus on the impact of oral lactulose supplementation at different doses over a longer period of time on blood glucose profile and gut microbiota.

ACKNOWLEDGEMENTS

The authors thank all subjects who took part in this clinical trial. Furthermore, the authors acknowledge Marlene Czarny (TechMedWriting Services, LLC, Jacksonville, Florida) and Christina Gatschelhofer (Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Austria) for reviewing and editing the manuscript.

REFERENCES

1 **Zhao J**, Frøkjaer JB, Drewes AM, Ejskjaer N. Upper gastrointestinal sensory-motor dysfunction in diabetes mellitus. *World J Gastroenterol* 2006; **12**: 2846-2857 [PMID: 16718808 DOI: 10.3748/wjg.v12.i18.2846]
2 **Zhao M**, Liao D, Zhao J. Diabetes-induced mechanophysiological changes in the small intestine and colon. *World J Diabetes* 2017; **8**: 249-269 [PMID: 28694926 DOI: 10.4239/wjd.v8.i6.249]
3 **Bytzer P**, Talley NJ, Hammer J, Young LJ, Jones MP, Horowitz M. GI symptoms in diabetes mellitus are associated with both poor glycemic control and diabetic complications. *Am J Gastroenterol* 2002; **97**: 604-611 [PMID: 11922554 DOI: 10.1111/j.1572-0241.2002.05537.x]
4 **el-Salhy M**. The possible role of the gut neuroendocrine system in diabetes gastroenteropathy. *Histol Histopathol* 2002; **17**: 1153-1161 [PMID: 12371143 DOI: 10.14670/HH-17.1153]
5 **Rayner CK**, Samsom M, Jones KL, Horowitz M. Relationships of upper gastrointestinal motor and sensory function with glycemic control. *Diabetes Care* 2001; **24**: 371-381 [PMID: 11213895 DOI: 10.2337/diacare.24.2.371]
6 **Ricci JA**, Siddique R, Stewart WF, Sandler RS, Sloan S, Farup CE. Upper gastrointestinal symptoms in a U.S. national sample of adults with diabetes. *Scand J Gastroenterol* 2000; **35**: 152-159 [PMID: 10720112 DOI: 10.1080/003655200750024317]
7 **Chandrasekharan B**, Anitha M, Blatt R, Shahnavaz N, Kooby D, Staley C, Mwangi S, Jones DP, Sitaraman SV, Srinivasan S. Colonic motor dysfunction in human diabetes is associated with enteric neuronal loss and increased oxidative stress. *Neurogastroenterol Motil* 2011; **23**: 131-138, e26 [PMID: 20939847 DOI: 10.1111/j.1365-2982.2010.01611.x]
8 **Piper MS**, Saad RJ. Diabetes Mellitus and the Colon. *Curr Treat Options Gastroenterol* 2017; **15**: 460-474 [PMID: 29063998 DOI: 10.1007/s11938-017-0151-1]
9 **Prasad VG**, Abraham P. Management of chronic constipation in patients with diabetes mellitus. *Indian J Gastroenterol* 2017; **36**: 11-22 [PMID: 27987136 DOI: 10.1007/s12664-016-0724-2]
10 **Cash BD**, Lacy BE. Systematic Review: FDA-Approved Prescription Medications for Adults With Constipation. *Gastroenterol Hepatol (N Y)* 2006; **2**: 736-749 [PMID: 28325992 DOI: 10.1136/gutjnl-2016-311835]
11 **Portalatin M**, Winstead N. Medical management of constipation. *Clin Colon Rectal Surg* 2012; **25**: 12-19 [PMID: 23449608 DOI: 10.1055/s-0032-1301754]
12 **Shakil A**, Church RJ, Rao SS. Gastrointestinal complications of diabetes. *Am Fam Physician* 2008; **77**: 1697-1702 [PMID: 18619079]
13 **DiPalma JA**. Current treatment options for chronic constipation. *Rev Gastroenterol Disord* 2004; **4** Suppl 2: S34-S42 [PMID: 15184812]
14 **Fritz E**, Hammer HF, Lipp RW, Högenauer C, Stauber R, Hammer J. Effects of lactulose and polyethylene glycol on colonic transit. *Aliment Pharmacol Ther* 2005; **21**: 259-268 [PMID: 15691300 DOI: 10.1111/j.1365-2036.2005.02244.x]
15 **Pontes FA**, Silva AT, Cruz AC. Colonic transit times and the effect of lactulose or lactitol in hospitalized patients. *Eur J Gastroenterol Hepatol* 1995; **7**: 441-446 [PMID: 7614107]
16 Panel on Dietetic Products N, Allergies. Scientific Opinion on the substantiation of health claims related to lactulose and decreasing potentially pathogenic gastro-intestinal microorganisms (ID 806) and reduction in intestinal transit time (ID 807) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA* 2010; **8**: 1806 [DOI: 10.2903/j.efsa.2010.1806]
17 **Bouhnik Y**, Neut C, Raskine L, Michel C, Riottot M, Andrieux C, Guillemot F, Dyard F, Flourié B. Prospective, randomized, parallel-group trial to evaluate the effects of lactulose and polyethylene glycol-4000 on colonic flora in chronic idiopathic constipation. *Aliment Pharmacol Ther* 2004; **19**: 889-899 [PMID: 15080850 DOI: 10.1111/j.1365-2036.2004.01918.x]
18 **Panesar PS**, Kumari S. Lactulose: production, purification and potential applications. *Biotechnol Adv* 2011; **29**: 940-948 [PMID: 21856402 DOI: 10.1016/j.biotechadv.2011.08.008]
19 **Steudle J**, Schön C, Wargenau M, Pauly L, Schwejda-Güttes S, Gaigg B, Kuchinka-Koch A, Stover JF. Blood glucose response after oral intake of lactulose in healthy volunteers: A randomized, controlled, cross-over study. *World J Gastrointest Pharmacol Ther* 2018; **9**: 22-30 [PMID: 30090645 DOI: 10.4292/wjgpt.v9.i3.22]
20 **Agiostratidou G**, Anhalt H, Ball D, Blonde L, Gourgari E, Harriman KN, Kowalski AJ, Madden P, McAuliffe-Fogarty AH, McElwee-Malloy M, Peters A, Raman S, Reifschneider K, Rubin K, Weinzimer SA. Standardizing Clinically Meaningful Outcome Measures Beyond HbA_{1c} for Type 1 Diabetes: A Consensus Report of the American Association of Clinical Endocrinologists, the

American Association of Diabetes Educators, the American Diabetes Association, the Endocrine Society, JDRF International, The Leona M. and Harry B. Helmsley Charitable Trust, the Pediatric Endocrine Society, and the T1D Exchange. *Diabetes Care* 2017; **40**: 1622-1630 [PMID: 29162582 DOI: 10.2337/dc17-1624]

21 **Garber AJ**, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, Dagogo-Jack S, Davidson MB, Einhorn D, Garber JR, Garvey WT, Grunberger G, Handelsman Y, Hirsch IB, Jellinger PS, McGill JB, Mechanick JI, Rosenblit PD, Umpierrez G, Davidson MH. AACE/ACE comprehensive diabetes management algorithm 2015. *Endocr Pract* 2015; **21**: 438-447 [PMID: 25877012 DOI: 10.4158/EP15693.CS]

22 **Heaton KW**, Radvan J, Cripps H, Mountford RA, Braddon FE, Hughes AO. Defecation frequency and timing, and stool form in the general population: a prospective study. *Gut* 1992; **33**: 818-824 [PMID: 1624166 DOI: 10.1136/gut.33.6.818]

23 **Teuri U**, Vapaatalo H, Korpela R. Fructooligosaccharides and lactulose cause more symptoms in lactose maldigesters and subjects with pseudohypolactasia than in control lactose digesters. *Am J Clin Nutr* 1999; **69**: 973-979 [PMID: 10232639 DOI: 10.1093/ajcn/69.5.973]

24 **Conn HO**, Bircher J. Hepatic encephalopathy: management with lactulose and related carbohydrates. *Medi-Ed Press* 1989 [DOI: 10.1002/hep.1840090624]

25 **Bianchi G**, Ronchi M, Marchesini G. Effect of lactulose on carbohydrate metabolism and diabetes mellitus. *Scand J Gastroenterol Suppl* 1997; **222**: 62-64 [PMID: 9145450 DOI: 10.1080/00365521.1997.11720721]

26 **Jenkins DJ**, Goff DV, Leeds AR, Alberti KG, Wolever TM, Gassull MA, Hockaday TD. Unabsorbable carbohydrates and diabetes: Decreased post-prandial hyperglycaemia. *Lancet* 1976; **2**: 172-174 [PMID: 73796 DOI: 10.1016/s0140-6736(76)92346-1]

27 **Day M**, Wills T, Coffey A. Constipation and the pros and cons of laxatives for older adults. *Nurs Res Care* 2014; **16**: 2-4 [DOI: 10.12968/nrec.2014.16.4.196]

28 **Waterfield J**. Laxatives: choice, mode of action and prescribing issues. *Nurse Prescri* 2007; **5**: 456-461 [DOI: 10.12968/npre.2007.5.10.27557]

29 **Yakabowich M**. Prescribe with care. The role of laxatives in the treatment of constipation. *J Gerontol Nurs* 1990; **16**: 4-11 [PMID: 2370432 DOI: 10.3928/0098-9134-19900701-04]

World Journal of Diabetes

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

World J Diabetes 2021 June 15; 12(6): 908-915

DOI: 10.4239/wjd.v12.i6.908

ISSN 1948-9358 (online)

META-ANALYSIS

Coffee consumption and risk of type 2 diabetes mellitus in Asians: A meta-epidemiological study of population-based cohort studies

Jong-Myon Bae

ORCID number: Jong-Myon Bae 0000-0003-3080-7852.

Author contributions: Bae JM designed the research study, performed the research; analyzed the data and wrote the manuscript.

Conflict-of-interest statement: The author declares no conflict of interests and no funding sources for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Manuscript source: Invited manuscript

Jong-Myon Bae, Department of Preventive Medicine, Jeju National University School of Medicine, Jeju-si 63243, South Korea

Corresponding author: Jong-Myon Bae, MD, PhD, Professor, Department of Preventive Medicine, Jeju National University School of Medicine, 102 Jejudaehak-ro, Jeju-si 63243, South Korea. jmbae@jejunu.ac.kr

Abstract

BACKGROUND

Previous systematic reviews have consistently reported that coffee consumption has a preventive effect on the occurrence of type 2 diabetes mellitus (T2DM). However, further evaluations between coffee consumption and the risk of T2DM in Asian populations are needed.

AIM

To conduct a meta-epidemiological study on systematic reviews evaluating the association between coffee consumption and the risk of T2DM in Asian people.

METHODS

The selection criterion was defined as a population-based prospective cohort study evaluating the association between coffee consumption and the risk of T2DM in Asian populations, reporting the adjusted relative risk (RR) and its 95% confidence interval (CI) for potential confounders. A fixed-effect model meta-analysis was applied to calculate the summary RR and its 95%CI in less than 50% of the I^2 value indicating the level of heterogeneity. A two-stage fixed-effects dose-response meta-analysis (DRMA) was performed to calculate the risk per unit dose (a cup per day).

RESULTS

A total of seven studies were selected in this meta-epidemiological study. The risk of T2DM in Asian populations was significantly reduced in the highest to the lowest dose group (summary RR = 0.73, 95%CI: 0.66-0.82; I^2 value = 0.0%). The DRMA showed that drinking one cup of coffee per day reduced the risk of T2DM in Asian populations by 8% (RR = 0.92, 95%CI: 0.90-0.95).

CONCLUSION

These findings support the conclusion that coffee consumption has a protective effect on the occurrence of T2DM in Asian men and women.

Specialty type: Endocrinology and metabolism

Country/Territory of origin: South Korea

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

Received: November 27, 2020
Peer-review started: November 27, 2020
First decision: December 20, 2020
Revised: December 23, 2020
Accepted: May 19, 2021
Article in press: May 19, 2021
Published online: June 15, 2021

P-Reviewer: Manfredini R, Yu EYW
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Li JH

Key Words: Coffee; Diabetes mellitus; Cohort studies; Meta-analysis; Systematic reviews

Core Tip: Previous systematic reviews have consistently reported that coffee consumption has a preventive effect on the occurrence of type 2 diabetes mellitus (T2DM). However, differences in coffee consumption habits by region could create heterogeneity. This research aimed to conduct a meta-epidemiological study on systematic reviews evaluating the association between coffee consumption and the risk of T2DM in Asian populations. From a total of seven Asian cohort studies, it was concluded that coffee consumption has a protective effect on the occurrence of T2DM in Asian men and women.

Citation: Bae JM. Coffee consumption and risk of type 2 diabetes mellitus in Asians: A meta-epidemiological study of population-based cohort studies. *World J Diabetes* 2021; 12(6): 908-915
URL: https://www.wjgnet.com/1948-9358/full/v12/i6/908.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i6.908

INTRODUCTION

The prevalence and incidence of type 2 diabetes mellitus (T2DM) has increased globally[1]. T2DM has a huge disease burden because of several cardiovascular, neuronal, renal, or ophthalmic complications[2].

Coffee consumption has been known to reduce the risk of T2DM since van Dam and Hu[3] reported it in 2005[3-8]. Further, Carlström and Larsson[8] reported an inverse association between coffee consumption and the risk of T2DM in Asian [summary relative risk (sRR) = 0.73, 95% confidence interval (CI): 0.64-0.82], European (sRR = 0.69, 95%CI: 0.62-0.75), and American (sRR = 0.74, 95%CI: 0.65-0.84) populations through a systematic review published in 2018. However, when analyzing the subgroups by geographical region, the I^2 values of the United States, Europe, and Asia were 73.8%, 46.8%, and 0.0%, respectively. Therefore, it can be inferred that the differences in coffee consumption habits by region could create heterogeneity. The Western Pacific region has the largest number of people with diabetes as reported by the International Diabetes Federation Diabetes Atlas[1]; therefore, further evaluations between coffee consumption and the risk of T2DM in Asian populations are needed.

In addition, the results of dose-response meta-analysis (DRMA) by sex as reported by Carlström and Larsson[8] showed that there was an indication of a stronger association in women (P for difference by sex = 0.03). Hence, it is necessary to determine whether there is a difference in the risk of T2DM between men and women in Asian populations according to coffee consumption.

Therefore, this research aimed to conduct a meta-epidemiological study on systematic reviews evaluating the association between coffee consumption and the risk of T2DM in Asian men and women.

MATERIALS AND METHODS

Selection strategies

The study subjects of the meta-epidemiological study were the original articles selected by previous systematic reviews[9,10]. Carlström and Larsson[8] selected five Asian cohort studies[11-15], with the latest year of publication in 2015[15]. Thus, it was necessary to include additional papers up to May 31, 2020. Hence, the "cited by" option provided by PubMed[16] was applied to make a list of papers citing the 31 papers selected in previous systematic reviews[4-8].

Papers were selected in the list according to the following criteria: (1) Population-based prospective cohort studies evaluating the association between coffee consumption and T2DM risk in the Asian population; and (2) Studies reporting the

adjusted RR and its 95%CI for potential confounders. Therefore, retrospective cohort studies, case-control studies, studies involving non-Asian populations, or studies that did not adjust for potential confounders were excluded.

Control of confounders

In the studies selected through the above selection processes, the level of adjusting smoking status (LAS) and level of adjusting alcohol intake (LAA) were evaluated by the levels reported by Thomas and Hodges[17], as smoking status and alcohol consumption are highly related to coffee consumption[17,18]. The low level was defined as adjusting for only status and the high level was defined as adjusting for intensity and status.

Statistical analysis

The RR and its 95%CI values adjusted for potential confounders in the highest *vs* lowest dose groups were extracted from each study for conducting a meta-analysis. The level of heterogeneity among the studies was evaluated as an I^2 value (%). A fixed-effect model was applied to calculate the sRR and its 95%CI in less than 50% of the I^2 value[19]. Subgroup analyses by sex (men, women), LAS (high, low), LAA (high, low), and family history of T2DM (FHX; yes, no) were conducted. The Egger test for small-study effects and funnel plots were performed to check for publication bias[20]. The non-parametric trim and fill analysis was performed to estimate the sRR reflecting publication bias[21].

In addition, a two-stage fixed-effects DRMA was performed to estimate the incidence risk *per* unit dose (a cup per day) considering the P value of the goodness-of-fit. The linear relationship was confirmed by testing the null hypothesis that the coefficients of the second and third splines were all equal to zero[22]. The fixed-effect meta-analysis, Egger's test, non-parametric trim and fill analysis, and two-stage fixed-effects DRMA were performed using metan, metabias, metatrim, and glst commands of STATA software, respectively (version 14.2, StataCorp, TX, United States). A P value of < 0.05 was considered statistically significant.

RESULTS

Final selection

A total of 560 papers cited 31 studies selected by previous systematic reviews until May 31, 2020. After applying the selection criteria, two new studies were selected[23,24]. Accordingly, a total of seven cohort studies were finally selected for the meta-analysis[11-15,23,24] (Figure 1). The distribution of these studies by nationality was four published in Japan, and one each in Singapore, Taiwan, and Korea. Seven studies had 12 cohorts by sex, including five men, five women, and two cohorts adjusted for sex. A total of 6348 T2DM cases developed in 141813 participants during the follow-up period. The results of evaluating LAS, LAA, and FHX in each study are shown in Figure 2.

Summary effect size

The risk of T2DM in Asian populations was significantly reduced in the highest coffee dose group compared to the lowest dose group (sRR = 0.73, 95%CI: 0.66-0.82; I^2 value = 0.0%) (Figure 2). As the funnel plot and Egger's test for small-study effects showed that a publication bias (P = 0.01) was present (Figure 3), a non-parametric trim and fill analysis was conducted. Furthermore, coffee consumption still prevented T2DM in Asian populations (sRR = 0.73, 95%CI: 0.54-0.91; P value of test for heterogeneity = 0.99).

The statistical significance of the preventive effect did not change in the results of subgroup analysis according to sex, LAS, LAA, or FHX (Table 1). The results of DRMA showed that drinking a cup of coffee per day reduced the risk of T2DM in Asian populations by approximately 8% (RR = 0.92, 95%CI: 0.90-0.95) with a linear relationship (Figure 4). In addition, men and women in Asia had a protective dose-response effect with statistical significance (Table 2).

DISCUSSION

The findings of this study can be summarized as follows: Coffee consumption could

Table 1 Summary relative risk and its 95% confidence intervals

Group		Summary relative risk (95% confidence intervals) [I-squared %]
All		0.73 (0.66-0.82) [0.0]
Sex	Men	0.76 (0.65-0.89) [0.0]
	Women	0.73 (0.59-0.90) [13.8]
Level of adjusting[1] tobacco smoking	High	0.72 (0.58-0.90) [21.0]
	Low	0.73 (0.63-0.84) [0.0]
Level of adjusting[1] alcohol drinking	High	0.72 (0.60-0.87) [0.0]
	Low	0.72 (0.60-0.87) [34.0]
Adjusting family history	Yes	0.72 (0.58-0.90) [21.0]
	No	0.73 (0.63-0.84) [0.0]

[1]High: Adjusted for intensity as well as status; Low: Adjusted for only status.

Table 2 Dose-response meta-analysis by the intaking unit (cup per day)

	Relative risk (95% confidence interval)	*P* value of heterogeneity	*P* value of non-linearity
All	0.92 (0.90-0.95)	0.42	0.31
Men	0.95 (0.91-0.98)	0.72	0.08
Women	0.89 (0.85-0.94)	0.40	0.56

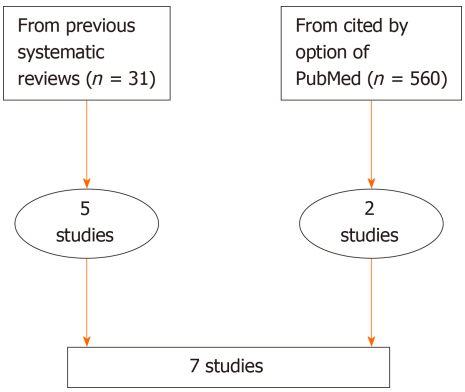

Figure 1 Flow chart of final selection.

decrease the occurrence of T2DM in Asian populations, and drinking a cup of coffee per day reduced the risk of T2DM in Asian population by approximately 8%.

Comparison with previous evidence

The results of this study consisting of 12 cohorts were consistent with the results of the study by Carlström and Larsson[8], which consisted of seven cohorts, in a meta-analysis comparing the highest to lowest coffee consumption. Women had a stronger association (11%) than men (5%) in the dose-response analysis per additional cup of coffee per day. In contrast to the results reported by Carlström and Larsson[8], this study showed statistically significant results in men. Hence, coffee consumption has a protective effect on the occurrence of T2DM in Asian men and women.

Natella and Scaccini[25] summarized the five effects of coffee in the modulation of diabetes mellitus risk and effects on glucose metabolism, thermogenic effects, antioxidant effects, anti-inflammatory effects, and chelating effects.

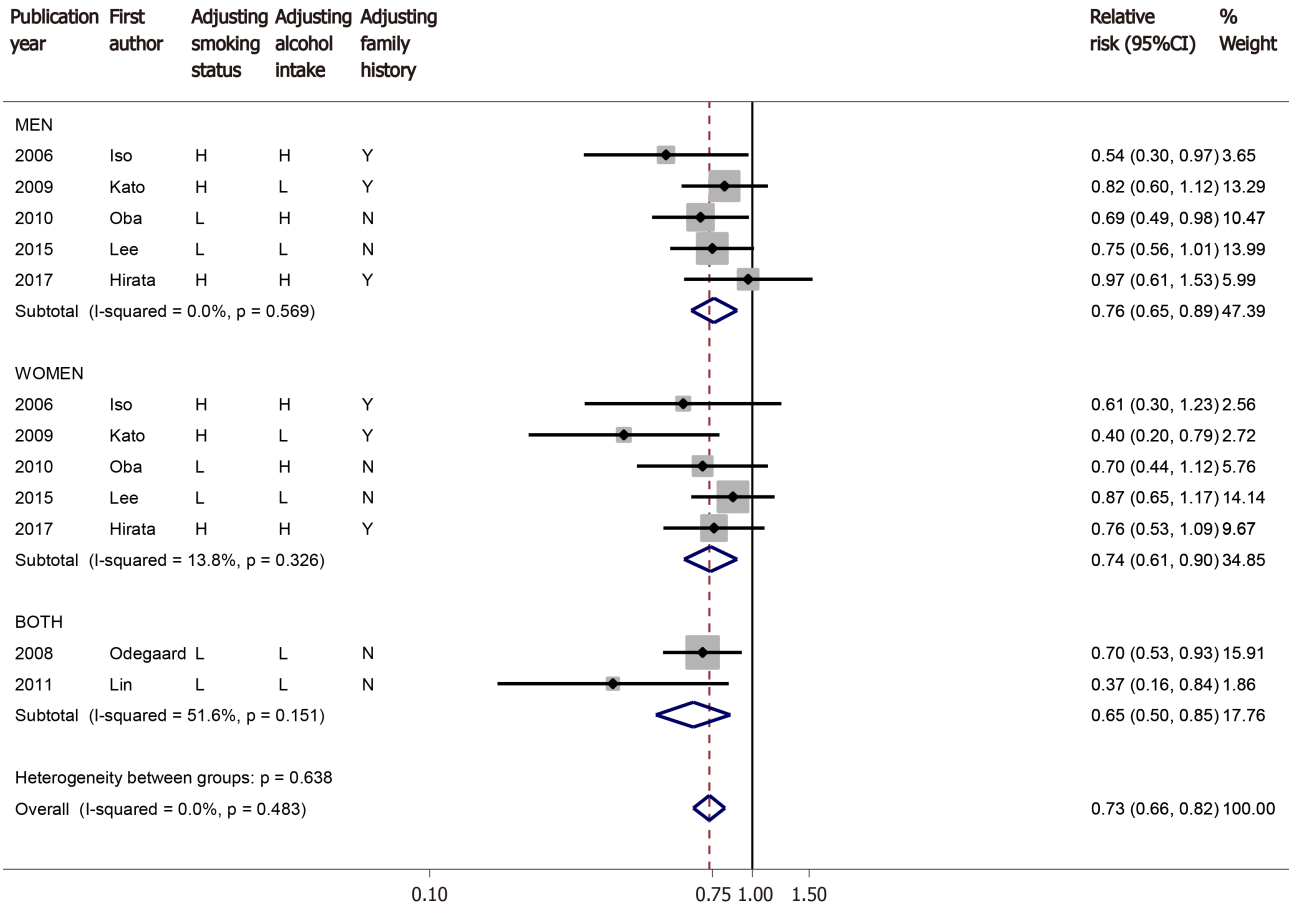

Figure 2 Forest plot to estimate summary relative risks by sex. CI: Confidence interval; H: High level; L: Low level; N: Not adjust; Y: Adjust.

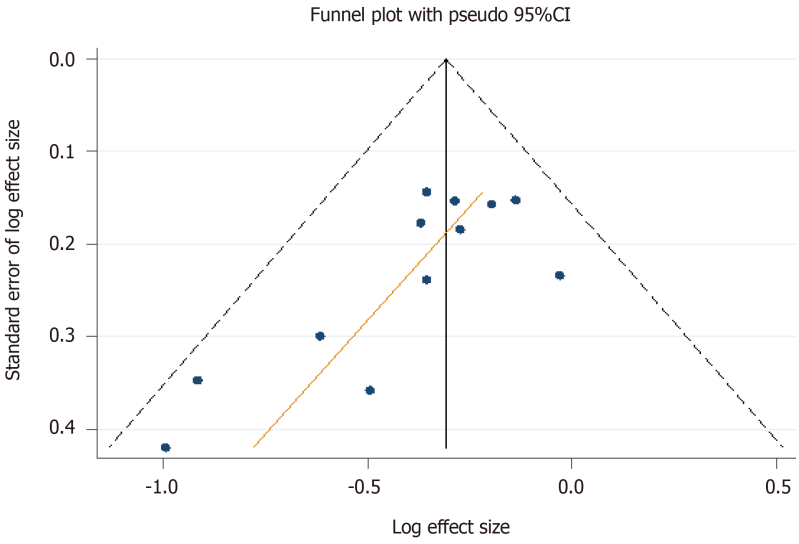

Figure 3 Funnel plot with Egger's test (*P* = 0.01).

Strengths

This meta-epidemiological study was able to find and include two cohort studies[23, 24], which should have been selected from existing systematic reviews. This was because they were cohort studies published before May 2017. It was re-confirmed that application of the "cited by" option of PubMed could be an effective search strategy [16].

As the Egger's test and funnel plot reported a publication bias, a non-parametric trim and fill analysis was conducted. Nevertheless, there was no change in the

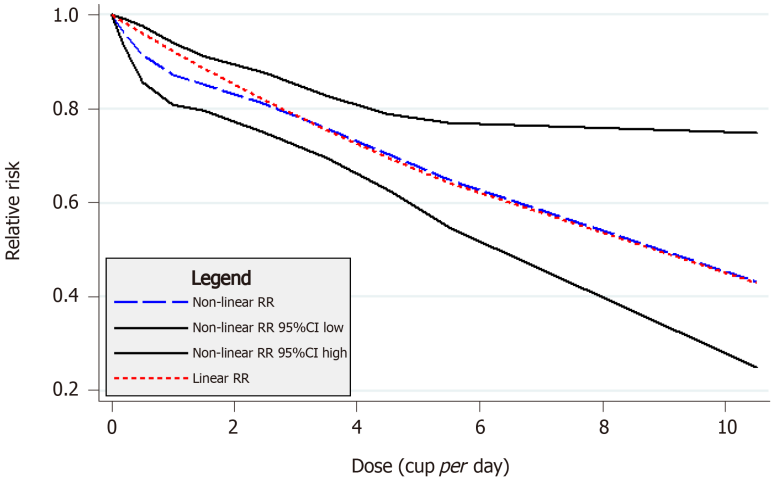

Figure 4 Dose-response meta-analysis in Asians. Relative risk and its low/high 95% confidence intervals. CI: Confidence interval; RR: Relative risk.

conclusion that coffee consumption reduced the risk of T2DM in Asian populations. As smoking and alcohol status would be highly related to coffee consumption, the LAS and LAA in each study were evaluated, and subsequently, subgroup analysis was performed. However, the preventive effect remained regardless of the LAS, LAA, and FHX. Therefore, coffee consumption had a protective effect on T2DM in Asian populations.

Limitations

The limitations of this meta-epidemiological study are the same as the limitations derived from the research design of prospective cohort studies that became the unit of meta-analysis.

Firstly, the information on coffee consumption and its quantity was usually obtained at the time of the cohort participation through a self-reported questionnaire. This should consider the possibility of measurement error[26]. The risk of T2DM decreased when the quantity of coffee consumption increased during the follow-up period, and the risk of T2DM increased when the quantity decreased[27]. Based on these findings, it can be inferred that the higher the coffee consumption, the more T2DM prevention, even if the coffee dose changes during the follow-up period.

In addition, when presenting the results obtained through follow-up, each cohort study had a different category of consumption and different references. Accordingly, the DRMA and meta-analysis using high *vs* low dose levels were conducted, and it was observed that coffee consumption prevented T2DM in Asian population.

CONCLUSION

This meta-epidemiological study confirmed that coffee consumption could prevent the occurrence of T2DM in Asian populations. Furthermore, the findings in the subgroup analyses by sex, LAS, LAA, and FHX showed the similar result consistently with statistical significance. In addition, the same results were obtained from the DRMA according to the daily consumption and the non-parametric trim and fill analysis in consideration of a publication bias. However, further studies are needed to investigate the preventive mechanism of coffee using a metabolomics study[28].

ARTICLE HIGHLIGHTS

Research background

The previous systematic reviews showed that an inverse association between coffee consumption and the risk of type 2 diabetes mellitus (T2DM).

Research motivation

While the differences in coffee consumption habits by region could create hetero-

geneity, further evaluations between coffee consumption and the risk of T2DM in Asian populations are needed.

Research objectives

The aimed to conduct a meta-epidemiological study to evaluate the association between coffee consumption and the risk of T2DM in Asian men and women.

Research methods

After selecting the studies meeting the selection criteria, a fixed-effect model meta-analysis and two-stage fixed-effects dose-response meta-analysis were performed.

Research results

Coffee consumption could decrease the occurrence of T2DM in the Asian population, and drinking a cup of coffee per day reduced the risk of T2DM in the Asian population by approximately 8%.

Research conclusions

This meta-epidemiological study concluded that coffee consumption could prevent the occurrence of T2DM in Asian populations.

Research perspectives

Further studies are needed to investigate the preventive mechanism of coffee using a metabolomics study.

REFERENCES

1 **Cho NH**, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018; **138**: 271-281 [PMID: 29496507 DOI: 10.1016/j.diabres.2018.02.023]

2 **Kim SH**, Lee ES, Yoo J, Kim Y. Predicting risk of type 2 diabetes mellitus in Korean adults aged 40-69 by integrating clinical and genetic factors. *Prim Care Diabetes* 2019; **13**: 3-10 [PMID: 30477970 DOI: 10.1016/j.pcd.2018.07.004]

3 **van Dam RM**, Hu FB. Coffee consumption and risk of type 2 diabetes: a systematic review. *JAMA* 2005; **294**: 97-104 [PMID: 15998896 DOI: 10.1001/jama.294.1.97]

4 **Neuenschwander M**, Ballon A, Weber KS, Norat T, Aune D, Schwingshackl L, Schlesinger S. Role of diet in type 2 diabetes incidence: umbrella review of meta-analyses of prospective observational studies. *BMJ* 2019; **366**: l2368 [PMID: 31270064 DOI: 10.1136/bmj.l2368]

5 **Huxley R**, Lee CM, Barzi F, Timmermeister L, Czernichow S, Perkovic V, Grobbee DE, Batty D, Woodward M. Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: a systematic review with meta-analysis. *Arch Intern Med* 2009; **169**: 2053-2063 [PMID: 20008687 DOI: 10.1001/archinternmed.2009.439]

6 **Ding M**, Bhupathiraju SN, Chen M, van Dam RM, Hu FB. Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: a systematic review and a dose-response meta-analysis. *Diabetes Care* 2014; **37**: 569-586 [PMID: 24459154 DOI: 10.2337/dc13-1203]

7 **Jiang X**, Zhang D, Jiang W. Coffee and caffeine intake and incidence of type 2 diabetes mellitus: a meta-analysis of prospective studies. *Eur J Nutr* 2014; **53**: 25-38 [PMID: 24150256 DOI: 10.1007/s00394-013-0603-x]

8 **Carlström M**, Larsson SC. Coffee consumption and reduced risk of developing type 2 diabetes: a systematic review with meta-analysis. *Nutr Rev* 2018; **76**: 395-417 [PMID: 29590460 DOI: 10.1093/nutrit/nuy014]

9 **Bae JM**. Meta-epidemiology. *Epidemiol Health* 2014; **36**: e2014019 [PMID: 25266419 DOI: 10.4178/epih/e2014019]

10 **Murad MH**, Wang Z. Guidelines for reporting meta-epidemiological methodology research. *Evid Based Med* 2017; **22**: 139-142 [PMID: 28701372 DOI: 10.1136/ebmed-2017-110713]

11 **Iso H**, Date C, Wakai K, Fukui M, Tamakoshi A; JACC Study Group. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Ann Intern Med* 2006; **144**: 554-562 [PMID: 16618952 DOI: 10.7326/0003-4819-144-8-200604180-00005]

12 **Odegaard AO**, Pereira MA, Koh WP, Arakawa K, Lee HP, Yu MC. Coffee, tea, and incident type 2 diabetes: the Singapore Chinese Health Study. *Am J Clin Nutr* 2008; **88**: 979-985 [PMID: 18842784 DOI: 10.1093/ajcn/88.4.979]

13 **Kato M**, Noda M, Inoue M, Kadowaki T, Tsugane S; JPHC Study Group. Psychological factors, coffee and risk of diabetes mellitus among middle-aged Japanese: a population-based prospective study in the JPHC study cohort. *Endocr J* 2009; **56**: 459-468 [PMID: 19270421 DOI: 10.1507/endocrj.k09e-003]

14 **Oba S**, Nagata C, Nakamura K, Fujii K, Kawachi T, Takatsuka N, Shimizu H. Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes in Japanese men and women. *Br J Nutr* 2010; **103**: 453-459 [PMID: 19818197 DOI: 10.1017/S0007114509991966]

15 **Lee JK**, Kim K, Ahn Y, Yang M, Lee JE. Habitual coffee intake, genetic polymorphisms, and type 2 diabetes. *Eur J Endocrinol* 2015; **172**: 595-601 [PMID: 25755232 DOI: 10.1530/EJE-14-0805]

16 **Bae JM**, Kim EH. Citation Discovery Tools for Conducting Adaptive Meta-analyses to Update Systematic Reviews. *J Prev Med Public Health* 2016; **49**: 129-133 [PMID: 27055549 DOI: 10.3961/jpmph.15.074]

17 **Thomas DR**, Hodges ID. Dietary Research on Coffee: Improving Adjustment for Confounding. *Curr Dev Nutr* 2020; **4**: nzz142 [PMID: 31938763 DOI: 10.1093/cdn/nzz142]

18 **Treur JL**, Taylor AE, Ware JJ, McMahon G, Hottenga JJ, Baselmans BM, Willemsen G, Boomsma DI, Munafò MR, Vink JM. Associations between smoking and caffeine consumption in two European cohorts. *Addiction* 2016; **111**: 1059-1068 [PMID: 26750569 DOI: 10.1111/add.13298]

19 **Harris RJ**, Bradburn MJ, Deeks JJ, Harbord RM, Altman DG, Sterne JAC. Metan: Fixed- and random-effects meta-analysis. *Stata J* 2008; **8**: 3-28 [DOI: 10.1201/9781420064759.ch1]

20 **Rücker G**, Carpenter JR, Schwarzer G. Detecting and adjusting for small-study effects in meta-analysis. *Biom J* 2011; **53**: 351-368 [PMID: 21374698 DOI: 10.1002/bimj.201000151]

21 **Steichen TJ**. Nonparametric trim and fill analysis of publication bias in meta-analysis. *Stata Tech Bull* 2000; **STB-57**: 8-14

22 **Orsini N**, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. *Stata J* 2006; **6**: 40-57

23 **Lin WY**, Xaiver Pi-Sunyer F, Chen CC, Davidson LE, Liu CS, Li TC, Wu MF, Li CI, Chen W, Lin CC. Coffee consumption is inversely associated with type 2 diabetes in Chinese. *Eur J Clin Invest* 2011; **41**: 659-666 [PMID: 21226707 DOI: 10.1111/j.1365-2362.2010.02455.x]

24 **Hirata A**, Ohnaka K, Tashiro N, Wang Z, Kohno M, Kiyohara C, Kono S, Takayanagi R. Effect modification of green tea on the association between rice intake and the risk of diabetes mellitus: a prospective study in Japanese men and women. *Asia Pac J Clin Nutr* 2017; **26**: 545-555 [PMID: 28429922 DOI: 10.6133/apjcn.042016.04]

25 **Natella F**, Scaccini C. Role of coffee in modulation of diabetes risk. *Nutr Rev* 2012; **70**: 207-217 [PMID: 22458694 DOI: 10.1111/j.1753-4887.2012.00470.x]

26 **Larsson SC**, Orsini N. Coffee Consumption and Risk of Dementia and Alzheimer's Disease: A Dose-Response Meta-Analysis of Prospective Studies. *Nutrients* 2018; **10** [PMID: 30322179 DOI: 10.3390/nu10101501]

27 **Bhupathiraju SN**, Pan A, Manson JE, Willett WC, van Dam RM, Hu FB. Changes in coffee intake and subsequent risk of type 2 diabetes: three large cohorts of US men and women. *Diabetologia* 2014; **57**: 1346-1354 [PMID: 24771089 DOI: 10.1007/s00125-014-3235-7]

28 **van Dam RM**. Can 'omics' studies provide evidence for causal effects of coffee consumption on risk of type 2 diabetes? *J Intern Med* 2018; **283**: 588-590 [PMID: 29611293 DOI: 10.1111/joim.12754]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com